Departement für Kleintiere, Klinik für Zoo-, Heim- und Wildtiere

der Vetsuisse-Fakultät Universität Zürich

Direktor: Prof. Dr. Jean-Michel Hatt

Arbeit unter wissenschaftlicher Betreuung von
Prof Dr. Marcus Clauss

Dietary abrasiveness and dental wear, growth and length measurements in rabbits (*Oryctolagus cuniculus*) and guinea pigs (*Cavia porcellus*)

Inaugural-Dissertation

zur Erlangung der Doktorwürde der

Vetsuisse-Fakultät Universität Zürich

vorgelegt von

Jacqueline Müller

Tierärztin

von Waldkirch, SG

genehmigt auf Antrag von

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Inhaltsverzeichnis

Vetsuisse Faculty University of Zurich (2014)

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Dietary abrasiveness and dental wear, growth and length measurements in rabbits (*Oryctolagus cuniculus*) and guinea pigs (*Cavia porcellus*)

Clinicians tend to believe that the ever-growing teeth of rabbits and rodents have a constant growth that needs to be worn down by the mastication of an appropriate diet. Experimental investigations on diet abrasiveness and tooth wear are rare. We investigated the effect of four different pelleted diets of increasing abrasiveness (due to both internal [phytoliths] and external abrasives [sand]) or whole grass hay fed for two weeks each in random order to 16 rabbits and 16 guinea pigs on incisor and premolar growth and wear, and incisor and cheek tooth length. Wear and tooth length differed between diets, with significant effects of both internal and external abrasives. Diet abrasiveness was linked to tooth length for all tooth positions; whole forage had an additional effect on upper incisors only. Tooth growth was strongly related to tooth wear and differed correspondingly between diets and tooth positions. Dental abnormalities were most distinct on the diet with sand. This study demonstrates that concepts of constant tooth growth requiring consistent wear are inappropriate, and that diet form (whole vs. pelleted) does not necessarily affect cheek teeth. Elucidating feedback mechanisms that link growth to tooth-specific wear represents a promising area of future research. The findings suggest that other factors than diet abrasiveness, such as mineral imbalances and in particular hereditary malocclusion, are more likely causes for dental problems observed in these species.

Abrasives, diet, tooth, lagomorph, rodent

Vetsuisse Fakultät Universität Zürich (2014)

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Abrasivität von Futtermitteln und Zahnabrieb, -wachstum und -längen bei Kaninchen (*Oryctolagus cuniculus*) und Meerschweinchen (*Cavia porcellus*)

Man vermutet, dass die Zähne von Kaninchen und Nagern ein konstantes Wachstum zeigen, welches durch das Kauen von geeignetem Futter abgerieben werden muss. Experimentelle Studien dazu sind rar. Hier wurde der Effekt von 4 unterschiedlichen pelletierten Futtermitteln mit steigender Abrasivität (via intrinsischer [Phytolithe] und extrinsischer Silikate [Sand]) und Grasheu auf Wachstum, Abrieb und Länge von Incisivi und Backenzähnen von 16 Kaninchen und 16 Meerschweinchen untersucht. Zahnabrieb und -wachstum variierten zwischen den Futtern, und intrinsische wie extrinisische Silikate hatten einen abrasiven Effekt. Die Abrasivität der Futter zeigte eine Verbindung mit der Zahnlänge bei allen Zahnpositionen; Grasheu hatte hingegen ausschliesslich einen Effekt auf die oberen Incisivi. Das Zahnwachstum korrelierte mit dem Abrieb und variierte mit der Zahnposition. Zahnveränderungen traten vor allem bei Fütterung von Sandpellets auf. Diese Studie zeigt, dass die Annahme eines konstanten Zahnwachstums, welches durch beständigen Abrieb im Gleichgewicht gehalten wird, unpassend ist, und dass die Form des Futtermittels (Heu vs. Pellets) die Backenzähne nicht unbedingt beeinflusst. Es ist noch mehr Forschungsarbeit notwendig, um den Feedbackmechanismus von Zahnwachstum und -abrieb aufzuklären.Unsere Resultate deuten an, dass andere Faktoren wie Mineralstoffimbalanzen oder genetische Gründe die Ursachen für die häufigen Zahnprobleme bei Kaninchen und Meerschweinchen sein könnten.

Abrasivität, Futtermittel, Zahn, Kaninchen, Nager

Für meine Eltern

RESEARCH ARTICLE

Growth and Wear of Incisor and Cheek Teeth in Domestic Rabbits (*Oryctolagus cuniculus*) Fed Diets of Different Abrasiveness

JOURNAL OF EXPERIMENTAL ZOOLOGY
PART A
ECOLOGICAL GENETICS AND PHYSIOLOGY
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ABSTRACT

Although patterns of tooth wear are crucial in palaeo-reconstructions, and dental wear abnormalities are important in veterinary medicine, experimental investigations on the relationship between diet abrasiveness and tooth wear are rare. Here, we investigated the effect of four different pelleted diets of increasing abrasiveness (due to both internal [phytoliths] and external abrasives [sand]) or whole grass hay fed for 2 weeks each in random order to 16 rabbits (*Oryctolagus cuniculus*) on incisor and premolar growth and wear, and incisor and cheek tooth length. Wear and tooth length differed between diets, with significant effects of both internal and external abrasives. While diet abrasiveness was linked to tooth length for all tooth positions, whole forage had an additional effect on upper incisor length only. Tooth growth was strongly related to tooth wear and differed correspondingly between diets and tooth positions. At 1.4–3.2 mm/week, the growth of cheek teeth measured in this study was higher than previously reported for rabbits. Dental abnormalities were most distinct on the diet with sand. This study demonstrates that concepts of constant tooth growth in rabbits requiring consistent wear are inappropriate, and that diet form (whole vs. pelleted) does not necessarily affect cheek teeth. Irrespective of the strong effect of external abrasives, internal abrasives have the potential to induce wear and hence exert selective pressure in evolution. Detailed differences in wear effects between tooth positions allow inferences about the mastication process. Elucidating feedback mechanisms that link growth to tooth-specific wear represents a promising area of future research. *J. Exp. Zool. 321A:283–298, 2014.* © 2014 Wiley Periodicals, Inc.

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Tooth Wear

Teeth are essential for most mammals. Dental adaptations to diets are well documented. In herbivorous mammals, various morphological adaptations (such as enamel ridge alignment) or chewing muscle size and physiological adaptations (such as rumination) have evolved to maximize chewing efficiency (Clauss et al., 2008; Fritz et al., 2009; Schwarm et al., 2009; Kaiser et al., 2010). Various adaptations are directed towards ensuring continuous tooth function in the presence of abrasion, most notably high tooth crowns (hypsodonty) (Damuth and Janis, 2011; Damuth and Janis, 2014), the ever-growing (hypselodont) teeth of many rodents and lagomorphs (Ungar, 2010), and increased enamel thickness (Rabenold and Pearson, 2011). Durable teeth are important for longevity and hence lifetime reproductive output (Skogland, '88; Kojola et al., '98; Loe et al., 2006; Veiberg et al., 2007).

Differences in the patterns of tooth wear have been described at the macroscopic and microscopic level as mesowear (Fortelius and Solounias, 2000) and microwear (Walker et al., '78), respectively, and microtexture (Ungar et al., 2003; Schulz et al., 2010). These patterns are interpreted in relation to the diets usually reported for the species in question (e.g., Rodrigues et al., 2009; Scott, 2012; Scott et al., 2012; Kaiser et al., 2013), but the resulting interpretations about the processes of wear remain mostly untested. Various analyses of tooth wear on populations with different diets or management practices (Ward and Mainland, '99; Mainland, 2000; Mainland, 2003a; Mainland, 2006; Clauss et al., 2007; Kaiser et al., 2009; Merceron et al., 2010; Taylor et al., 2014) help formulate such testable concepts of tooth wear. Controlled feeding experiments that test hypotheses about tooth wear on different diets have, in contrast, only been rarely reported so far (Mainland, 2003b; Schulz et al., 2013; Solounias et al., 2014).

Processes of tooth wear are still not well understood. "Wear" of dental tissue can be caused by both tooth-to-tooth contact, that is, attrition, and by abrasion due to internal abrasives, such as phytoliths in grasses, or external abrasives such as dust or grit (Butler, '72; Fortelius, '85; Kaiser et al., 2013). However, to what degree internal and external abrasives cause dental tissue loss is a matter of ongoing debate (Mainland, 2003a; Sanson et al., 2007; Damuth and Janis, 2011; Lucas et al., 2013; Erickson, 2014; Rabenold and Pearson, 2014). The degree of hypsodonty in herbivores is linked to the excretion (and hence intake) of abrasive elements in their faces (Hummel et al., 2011), but clarification of whether these elements represent internal or external abrasives is still lacking. That the ingestion of external abrasives induces wear was demonstrated in several studies (Healy and Ludwig, '65; Ludwig et al., '66; Healy et al., '67; Mainland, 2003a) and is usually not debated, but the contribution of internal abrasives remains largely untested.

Ever-Growing Teeth

The incisors of rodents and lagomorphs, and in some species also the cheek teeth, are ever-growing, which is an evident adaptation to compensate for tooth wear (Rensberger, '75; Rensberger, '86; Williams and Kay, 2001; Schmidt-Kittler, 2002). It is obvious that the rates of wear and growth must match in ever-growing teeth in order to maintain proper occlusion (Schmidt-Kittler, 2002; Ungar, 2010). However, to what degree growth actually responds to wear, and whether a certain growth occurs at a fixed rate that cannot be modified, is mostly unexplored. In veterinary clinical practice, and in common veterinary textbooks, constant growth is assumed that will lead to dental abnormalities if wear is insufficient (Crossley, 2000; Harkness et al., 2010). This concept is supported by findings in abnormal pet rabbits where the incisor teeth are not in occlusion: normal tooth wear cannot occur, and the incisors grow uncontrolled, with the maxillary incisors typically curling inward into the oral cavity or flaring out laterally, and the mandibular incisors protruding from the mouth (Van Caelenberg et al., 2008; Harcourt-Brown, 2009).

However, common sense suggests the existence of some regulatory mechanism that matches growth to abrasion, because free-ranging animals will face a variety of resources that are not identical in their abrasiveness, and will also undergo a variety of metabolic states that require different levels of food intake. For example, during hibernation, tooth growth is decreased distinctively in ground squirrels (*Spermophilus tridecemlineatus*) (Sarnat and Hook, '42). In beavers (*Castor canadensis*), growth rates of incisor teeth vary between seasons, being higher during summer and lower during winter (Rinaldi and Cole, 2004). Actually, in a study on tooth wear and growth in pet rabbits (Wolf and Kamphues, '95; Wolf and Kamphues, '96), incisor growth rates varied on different diets, apparently compensating for differences in tooth wear, but this flexibility was not emphasized.

An important aspect of a putative regulatory mechanism for tooth growth is that beyond the findings on variation with season or diet, this mechanism must be tooth-specific, because wear will differ between teeth of different positions, especially between incisors and cheek teeth. In the veterinary literature, growth rates of rabbit teeth are given as 1.3–3.0 mm/week for incisors (Wolf and Kamphues, '96) and 2.0–3.0 mm/month for cheek teeth (Meredith, 2007; Lord, 2011; Schumacher, 2011), with the implication that this growth needs to be matched by constant wear (induced by an appropriate diet) to avoid overgrowth.

Conflicts of interest: None.

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Evidence for tooth-specific growth regulation comes from examples in rats and rabbits where a single incisor was broken off and showed an increased growth rate as compared to its contralateral neighbor that was unbroken and hence in continuous occlusion with its antagonist (Schour and Medak, '51; Ness, '56).

Rabbits as Model Animals

Rabbits are attractive model animals to study tooth wear and growth because they are natural herbivores accepting a variety of feeds, and are comparatively easy to maintain. Furthermore they have continuously growing incisors and cheek teeth, which can be manipulated for macroscopic inspection as well as for computed tomography (CT), and their dental health has been studied extensively in the veterinary literature (Meredith, 2007; Capello and Cauduro, 2008; Van Caelenberg et al., 2010; Van Caelenberg et al., 2011; Jekl and Redrobe, 2013). Dental problems are one of the most important conditions for presenting pet rabbits to veterinary clinics, with frequencies for dental disease in rabbits ranging from 6.7% (Mosallanejad et al., 2010), to 14% (Langenecker et al., 2009), 30% (Mullan and Main, 2006), and even 38.1% (Jekl et al., 2008). Although these surveys show how significant dental problems are, the etiology of this disease complex is still not fully understood.

Malocclusion is the common denominator of dental problems in rabbits, but the reasons for malocclusion to occur in the first place are debated (Jekl and Redrobe, 2013). They are divided into congenital and acquired causes (Lennox, 2008). Congenital or hereditary tooth abnormalities are often diagnosed in younger animals. The most common condition is incisor overgrowth. This may be due to maxillary brachygnathia or brachycephalism, and is particularly seen in dwarf breeds (Crossley, '95). The underlying cause is an autosomal recessive gene (Harkness et al., 2010). In most cases the incisor teeth are not in occlusion and the mandibular incisors deviate labially from the maxillary ones.

Acquired causes may include traumatic injuries that lead to malocclusion (Capello, 2008), but are mainly related to diet, and to management factors that determine exposure to UVB light (Jekl and Redrobe, 2013). Dietary mineral imbalances, or lack of exposure to UVB, may lead to metabolic bone disease, which may impair occlusion due to osteodystrophy of the supporting bone and dental tissue malformation (Harcourt-Brown, '95). Experimentally, minerally imbalanced diets led to cheek tooth elongation and enamel hypoplasia in degus (*Octodon degus*) (Jekl et al., 2011a,b). The other major dietary factor considered responsible for dental abnormalities are easily digestible (i.e., low-fiber) diets that limit the absolute food intake, because energetic requirements are met by small amounts of such diets, leading to insufficient chewing activity and hence insufficient attrition (Wolf and Kamphues, '96; Crossley, 2003; Meredith, 2007; Harkness et al., 2010; Lord, 2011). Feeding pet rabbits dried forages as the staple diet item is therefore—amongst other reasons—recommended (Boehmer and Koestlin, '88; Clauss, 2012). Whether dietary abrasiveness itself, in addition to the effect of food intake and chewing activity, is also important has so far not been investigated. Dental problems are epidemiologically related to the use of low-fiber, energy-dense feeds that are, however, also often minerally imbalanced or leave the animals the choice of selecting minerally imbalanced ingredients (Harcourt-Brown, '96; Mullan and Main, 2006). Jekl and Redrobe (2013) suggest that a combination of metabolic bone disease and insufficient wear or lack of chewing action is responsible for dental disease in pet rabbits.

Aims of This Study

We aimed at investigating the effect of diet on dental wear in rabbits using a set of four complete, pelleted feeds varying in the amount of internal and external abrasives. The pelleted diets were based on lucerne (*Medicago sativa*), which naturally contains very low levels of internal abrasives (Wöhlbier, '83), grass, which contains higher levels of internal abrasives, and grass with the addition of rice hulls, which contain again higher levels (Wöhlbier, '83). The fourth pelleted diet included grass and rice hulls (internal abrasives), and additionally sand (external abrasives). To avoid differences in the total amount ingested (and hence chewing activity) between these pelleted diets, they were formulated to be isocaloric and isonitrogenic (using an indigestible, non-silicacious filler and soybean meal). The following hypotheses guided our approach:

1. Tooth growth compensates for wear; therefore we expect tooth length (TL) to be relatively constant across diets and growth tightly correlated with wear.
2. Small, detectable differences in wear between diets reflect diet abrasiveness and/or chewing activity.
3. Functional differences between incisors and cheek teeth lead to different wear and growth on different diets, that is,
   a. incisors are worn more heavily when feeding whole hay that needs more gnawing as compared to pellets;
   b. cheek teeth, with a chewing action more independent from whether the diet is offered whole or pelleted, are worn more heavily with increasing dietary abrasiveness; external abrasives (sand) lead to a gradient in wear along the maxillary cheek tooth row whereas increased internal abrasives (phytoliths in rice hulls) do not lead to such a gradient (Taylor et al., 2013).
4. Abnormal tooth wear will occur more frequently with excessive external abrasives (sand), and (according to 3b) affect the cheek teeth according to their position in the tooth row (anterior ones more affected).

MATERIALS AND METHODS

Animals and Diets

This experiment was approved of by the Cantonal Veterinary Office in Zurich, Switzerland (no. 80/2012). Sixteen female New

Zealand White rabbits (mean starting body mass 2.75 ± 0.16 kg; starting age approximately 7 months) were kept individually in hutches (1.00 × 0.75 m^2) on wood shavings and with plastic hides without other gnawing opportunities except their diet. Water was provided ad libitum. After a week of acclimatization the rabbits were randomly placed on one of five different diets for a 2-week-period. Diets consisted either of a grass hay fed as whole forage, or of complete pelleted diets formulated to be isocaloric and isonitrogenic, but of increasing abrasiveness from lucerne pellets (L), grass pellets (G), grass and rice hull pellets (GR), and grass and rice hull pellets with an addition of sand (GRS) (Table 1). Pellets were uniform across diets, of approximately 1 cm length and 4 mm diameter, requiring breaking with incisors before they could be chewed. Diets were fed ad libitum. Food intake was measured on a daily basis by weighing food offered and leftover. Rabbits were weighed at the end of each week, and the difference in body mass to the previous measure expressed in %. Food intake rate was measured as a proxy for chewing intensity on the different diets by timing the duration it took each rabbit to ingest 10 g (as fed). After 14 days, a new, again randomly selected diet was introduced to each rabbit with a 2-day-interval of mixing the old and the new diet to prevent digestive problems due to abrupt diet change. At the end of the experiment, all animals had received all diets. To evaluate digestibility of the different diets, total feces from each animal were collected and weighed for three consecutive days in the second week of every 2-week-interval. Representative samples of feeds, leftovers (in the case of hay), and feces were taken, dried at 60°C to constant weight, and prepared for further analyses by grinding. Protein, detergent fibers and acid detergent insoluble acid (ADIA) were measured using standard procedures (Van Soest et al., '91; VDLUFA, 2007, method 4.1.2, Dumas method; Hummel et al., 2011). A sample of the sand included in GRS was analyzed for mean particle size by wet sieving according to Fritz et al. (2012).

Dental Measurements

In the dental nomenclature used here, I^1 and I_1 denote the upper and lower incisor, respectively, maxillary (upper) cheek teeth are

Table 1. Composition of different complete pelleted diets (lucerne L, grass G, grass and rice hulls GR, grass and rice hulls and sands GRS) and grass hay (H).

	L	G	GR	GRS	H
Ingredients					
Lucerne meal (%)	60.0	–	–	–	–
Grass meal (%)	–	60.0	64.8	64.8	–
Rice hulls (%)	–	–	20.0	20.0	–
Sand[a] (%)	–	–	–	5.0	–
Pure lignocellulose (%)	33.8	27.4	5.0	–	–
Soybean meal (%)	–	7.0	5.0	5.0	–
Molasses (%)	3.0	3.0	3.0	3.0	–
Lignobond (%)	2.0	2.0	2.0	2.0	–
Soy oil (%)	1.0	0.4	–	–	–
Mineral/vitamin premix (%)	0.2	0.2	0.2	0.2	–
Dry matter (% as fed)	91.4	91.9	91.8	92.2	90.8
Nutrient composition (g/kg DM)					
Total ash	79	64	75	130	104
Crude protein	102	90	97	85	109
aNDFom[b]	578	600	487	459	579
ADFom[c]	434	403	322	299	354
ADL[d]	131	110	74	65	52
ADIA[e]	05	16	24	77	38
Dry matter digestibility (%)	39.7 ± 9.3	34.3 ± 8.1	41.2 ± 5.7	40.7 ± 11.1	45.1 ± 4.1

[a]Sand for playgrounds, grain size 0–1 mm, REDSUN garden products B.V., Heijen, Denmark; mean particle size measured by sieve analysis as dMEAN (Fritz et al., 2012) of 0.233 mm.
[b]aNDFom neutral detergent fiber, determined using amylase and ash corrected.
[c]ADFom acid detergent fiber, ash corrected.
[d]ADL acid detergent lignin ash corrected.
[e]ADIA acid detergent insoluble ash (a measure for abrasives).

denoted by capital letters (P or M for premolars and molars, respectively, i.e., P2-M3), mandibular (lower) cheek teeth by lower case letters (p or m, i.e., p2-m3) (Thenius, '89). At the beginning of each 2-week-interval, the rabbits' teeth were burred and CT of the head was carried out, and a final CT was taken at the end of the last feeding period. For these procedures the animals were placed under general anesthesia. The animals were sedated with 0.5–1.5 mg/kg midazolam (Dormicum®, Roche AG, Reinach, Switzerland) intramuscularly and anesthesia was induced and maintained with isoflurane administered in oxygen using a facemask 20–30 min later. During anesthesia, punctual marks of the size of approximately 1 mm were burred on the labial side of the I^1 and I_1 of both sides (total of 4 marks), and on the mesial side of the first lower cheek teeth (p3, cf. Fig. 1a) of either side (total of 2 marks) using a diamond burr (KaVo, EWL, Leutkirch i. A., Germany). Visible tooth crown length, and distances between gingival margin and mark (to assess tooth growth) and between mark and tooth edge (to assess tooth wear) of the I^1 and I_1 were measured (Wolf and Kamphues, '95) using an electronic digital caliper (Technocraft® Allchemet AG, Bäretswil, Switzerland, precision 0.01 mm). For the p3, because calipers could not be introduced far enough into the oral cavity, the same measures were estimated using a periodontal probe as a scale. Directly after burring, CT images of the head of each rabbit were obtained. The rabbits' recovery from anesthesia was uneventful in all cases and they usually started eating again two hours later. To monitor wear and growth of incisor teeth, caliper measurements were repeated a week later under manual restraint. Because the visible crowns of the p3 were smaller than those of the incisor teeth and the marks tended to wear off sooner, p3 were monitored more closely under manual restraint every 3–4 days. This was done by digital photography of the mesial side of each p3, taken with a portable endoscopic camera (Envisioner Medical Technologies, Inc., Rockville, Maryland), a LED-battery light source (Karl Storz GmbH, Tuttlingen, Germany) and the use of a rigid telescope (170°, 23 cm × 2.0 mm, Richard Wolf GmbH, Knittlingen, Germany) guided through the metal cone of an otoscope (Fig. 1a). Again, TL and distances from gingival margin to the mark and from the mark to the tip of the tooth were estimated. All manual measurements were taken by the same examiner (J.M.).

Computed Tomography and Evaluation

CT scans (Fig. 1b) were obtained using a 16 slice, spiral CT-scanner (Philips Brilliance 16, Philips Healthcare, Zurich, Switzerland). Images were acquired at 120 KV, 117 mA, a 10 cm FOV with a slice thickness of 1 mm. The rabbit was positioned in ventral recumbency on the CT table to obtain transverse sections. The original data were reconstructed with a soft-tissue and a bone algorithm and was reviewed using a bone window setting (window width = 3,814 HU, window level 594 HU) and a soft tissue window setting (window width = 270 HU, window level = 100 HU). CT images of the heads of all animals were investigated using OsiriX® software (PixmeoSarL, Bernex, Switzerland). Total lengths of all teeth were measured except for the peg teeth and the M3 and m3. Incisor teeth were measured in the sagittal plane, cheek teeth in the coronal plane with the help of an open polygon, a function that made it possible to measure curved structures. For each tooth the total length of the lingual and the buccal side was measured using this function as a curved structure from the base to the apex of the tooth. On CT images special attention was given to the occlusal surface, and it was noted whether spurs existed or if the tooth angle (TA) of the occlusal surface was abnormally sloped. Tooth spurs (TSP) were categorized from no spurs, slight, moderate or severe spurs (Scores 0–3, see Supplementary Material for examples of all scores used in this study), as spurs were too small for exact measurements. It was noted whether the occlusal surface (TSF) of each cheek tooth was either horizontal (Score 0), convex (Score −1) or concave (Score 1). The tooth surface angle (TA) was scored as horizontal (Score 0), sloped to the buccal side (Score −1) or sloped to the lingual side (Score 1). Additionally the dentition was checked for signs of waviness in the sagittal plane ("stepmouth") and again categorized from no signs, slight, moderate or severe signs of waviness (Scores 0–3). All CT scans were evaluated by the same examiner (J.M.) who was blinded to the diet that the respective scans represented.

Statistics

Data were analyzed using mixed-effects linear models (MELMs), accounting for repeated measurements from the same individuals by including Individual as a random effect to ensure correct error terms were being compared. Initially, we compared the general nutritional status of rabbits across diet treatments, including Diet as a main effect, and evaluating several response variables: body mass at the end of the relevant trial period; the relative (as a percentage of initial body mass) change in body mass over the relevant trial period (body $\text{mass}_{\text{change}(\%)}$); dry matter intake (DMI); acid detergent insoluble ash intake (ADIAI); dry matter digestibility (DMD); and intake rate (speed; measured as the time, in minutes, required to eat 10 g of food). Except for intake rate and DMD (which were only measured in the second week of each diet regime), we also included the Week on a particular diet treatment (1 or 2), and the Previous diet treatment (body mass variables only), as well as interactions between these terms with Diet, to account for autocorrelation and other temporal effects. We then assessed diet effects on TL, growth, and wear using similar MELMs, except that for TL (week 2 only) we also included Tooth as a main effect (as well as its interaction with Diet) to test for differences along the cheek tooth row. We also analyzed wear and growth responses to ADIAI and intake rate independently of the specific diet treatment, by replacing Diet with continuous quantitative variables—that is, wear as an effect on growth, and ADIAI (abrasiveness of diet) and intake rate, respectively, as effects on wear. Finally, to check for diet effects and tooth row position on dental abnormalities (hypothesis 4), we used MELMs

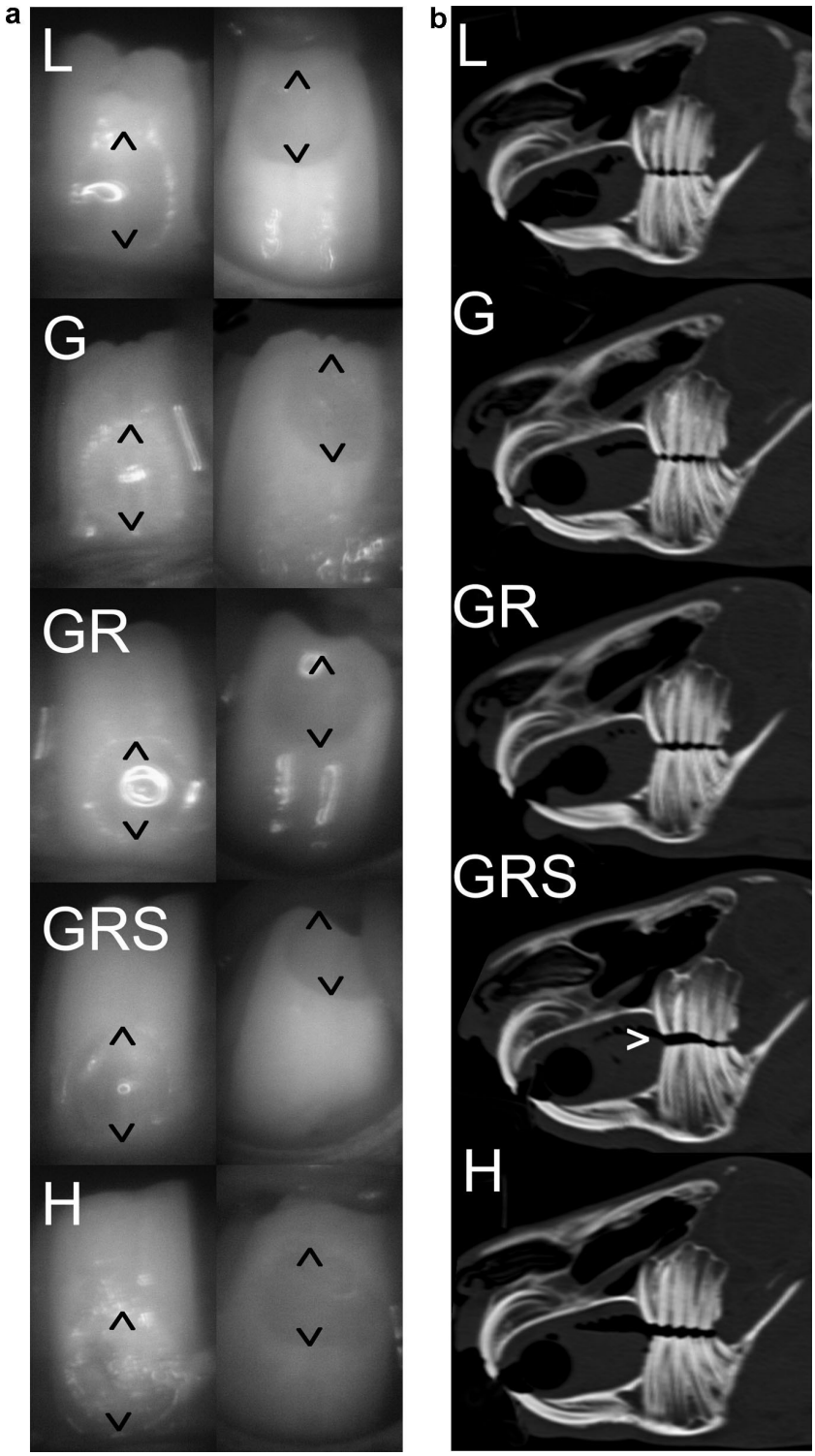

Figure 1. (a) Burr mark on the first lower premolar (p3) at the beginning and after 7 days on a given diet; borders of mark indicated by black arrows, (b) cranial CT scans after 2 weeks on a given diet in rabbits (*Oryctolagus cuniculus*) fed pelleted diets of lucerne (L), grass (G), grass and rice husks (GR), grass, rice husks and sand (GRS), and whole grass hay (H). Note the increased waviness of the cheek teeth on GRS (white arrow).

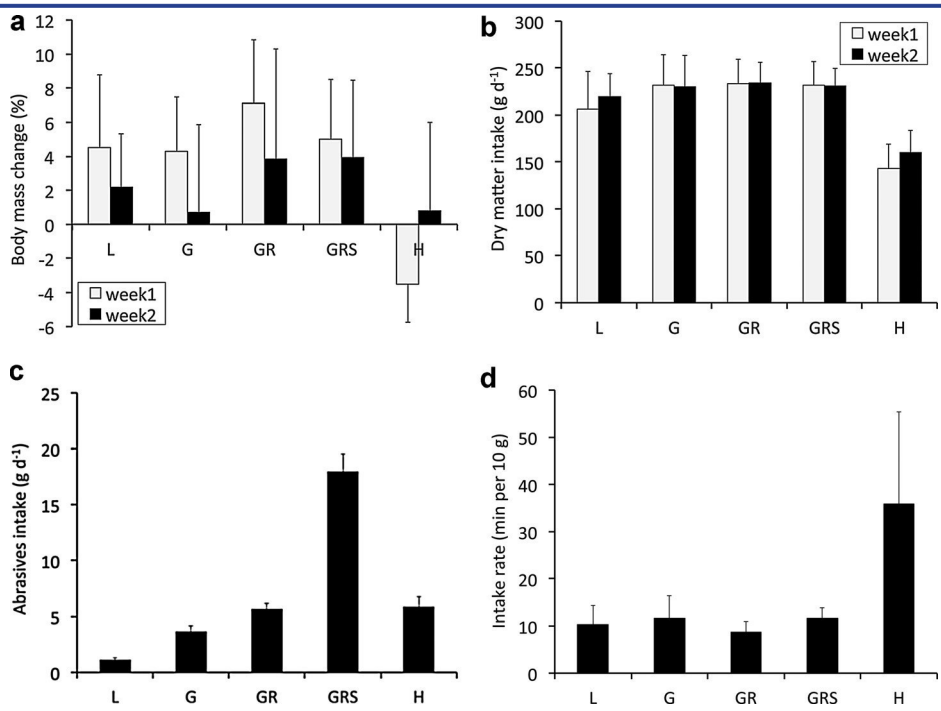

Figure 2. (a) Body mass change (in %), (b) dry matter intake (in g/day), (c) abrasives intake (measured as acid detergent insoluble ash, in g/day), and (d) intake rate (measured as time required to eat 10 g of diet) in rabbits (*Oryctolagus cuniculus*) fed pelleted diets of lucerne (L), grass (G), grass and rice husks (GR), grass, rice husks and sand (GRS), and whole grass hay (H). For (a) and (b), data are given individually for the first and second week on the respective diet.

with spurs (TSP), tooth surface (TSF), and TA scores, respectively, as well as waviness, as dependent variables, and Diet and Tooth (the latter not in assessments of Waviness) as main and interactive effects. However, for these models we excluded teeth for which abnormality scores were not different from zero, based on one-sample *t*-tests.

Data for incisors and cheek teeth, and for upper and lower teeth, were analyzed in separate MELMs. In cases where interaction terms were not significant ($P > 0.05$), these were removed and the relevant models repeated without them. Bonferroni post hoc tests were used for multiple comparisons where necessary. All analyses were carried out in STATISTICA version 8 (Statsoft_Inc., 2007).

RESULTS

General Diet Effects

Rabbit body mass differed across diet treatments, with lower means for rabbits on H than on other diets ($F_{4,119} = 10.178$, $P < 0.0001$ for body mass; and $F_{4,115} = 8.319$, $P < 0.0001$ for body $\text{mass}_{\text{change}(\%)}$). However, this effect was limited to the first week after a switch to any new diet treatment, as indicated by the significant interaction between Diet and Week for body $\text{mass}_{\text{change}(\%)}$ ($F_{4,115} = 3.646$, $P < 0.01$) (Fig. 2a), and the fact that body mass did not differ between weeks 1 and 2 ($F_{1,119} = 0.592$, $P = 0.443$). An animal's previous diet had no effect on body mass variables ($F_{4,119} = 0.192$, $P = 0.942$ for body mass; $F_{4,115} = 1.286$, $P = 0.280$ for body $\text{mass}_{\text{change}(\%)}$), further indicating that body mass changes occurred as short-term responses to switches in diet regime, but thereafter stabilized.

The DMI, ADIAI, DMD, and feeding rate of rabbits also differed across diet regimes ($F_{4,123} = 57.467$, $P < 0.0001$; $F_{4,123} = 1814.718$, $P < 0.0001$; $F_{4,59} = 3.719$, $P < 0.01$; and $F_{4,47} = 22.545$, $P < 0.0001$, respectively). DMI was lower for H than all other diets (Bonferonni post hoc $P < 0.0001$), and low for L relative to pelleted diets, although this latter difference was not always significant ($P = 0.089$ for G; $P = 0.021$ for GR; and $P = 0.068$ for GRS) (Fig. 2b). As expected, a trend of increasing ADIAI was found across diets with increasing levels of abrasiveness, with lowest ADIAI for L, followed by G, then GR, and finally highest for GRS ($P < 0.0001$ in all cases) (Fig. 2c). On GRS, approximately 70% of ADIAI was due to external abrasives (sand). On H, rabbits experienced similar ADIAI levels as for GR ($P = 0.999$). These patterns for DMI and ADIAI were consistent across both weeks of feeding trials (week effect $F_{1,123} = 2.158$ and 1.424, $P = 0.144$ and

0.235, respectively). The effect of diet on DMD (Table 1) probably occurred because of higher digestibility of H than G ($P < 0.01$). Rabbits required more time to ingest H than all other diets ($P < 0.0001$ in all cases) (Fig. 2d).

Effects on Tooth Length, Wear, and Growth

Diet had a significant influence on TL ($F_{4,60} = 9.203$, $P < 0.0001$ for I^1; $F_{4,360} = 65.391$, $P < 0.0001$ for upper cheek teeth; $F_{4,297} = 38.834$, $P < 0.0001$ for lower cheek teeth) (Fig. 3a,c,e). Only in the I_1 ($F_{4,60} = 0.944$, $P = 0.445$) did diet have no influence on TL (Fig. 3a). In I^1 TL was lower for H than for other diets (Bonferroni post hoc $P < 0.0001$ to < 0.001), whereas in cheek teeth TL for GRS was lower compared with other diets ($P < 0.0001$). TL was also lower for H and GR compared with L and G diets in upper and lower cheek teeth ($P < 0.0001$–0.032), indicating an effect of an increased level of internal abrasives.

TL was not influenced by the previous diet ($F_{4,119} = 2.429$, $P = 0.052$ for I^1; $F_{4,104} = 0.575$, $P = 0.681$ for I_1; $F_{4,72} = 2.111$,

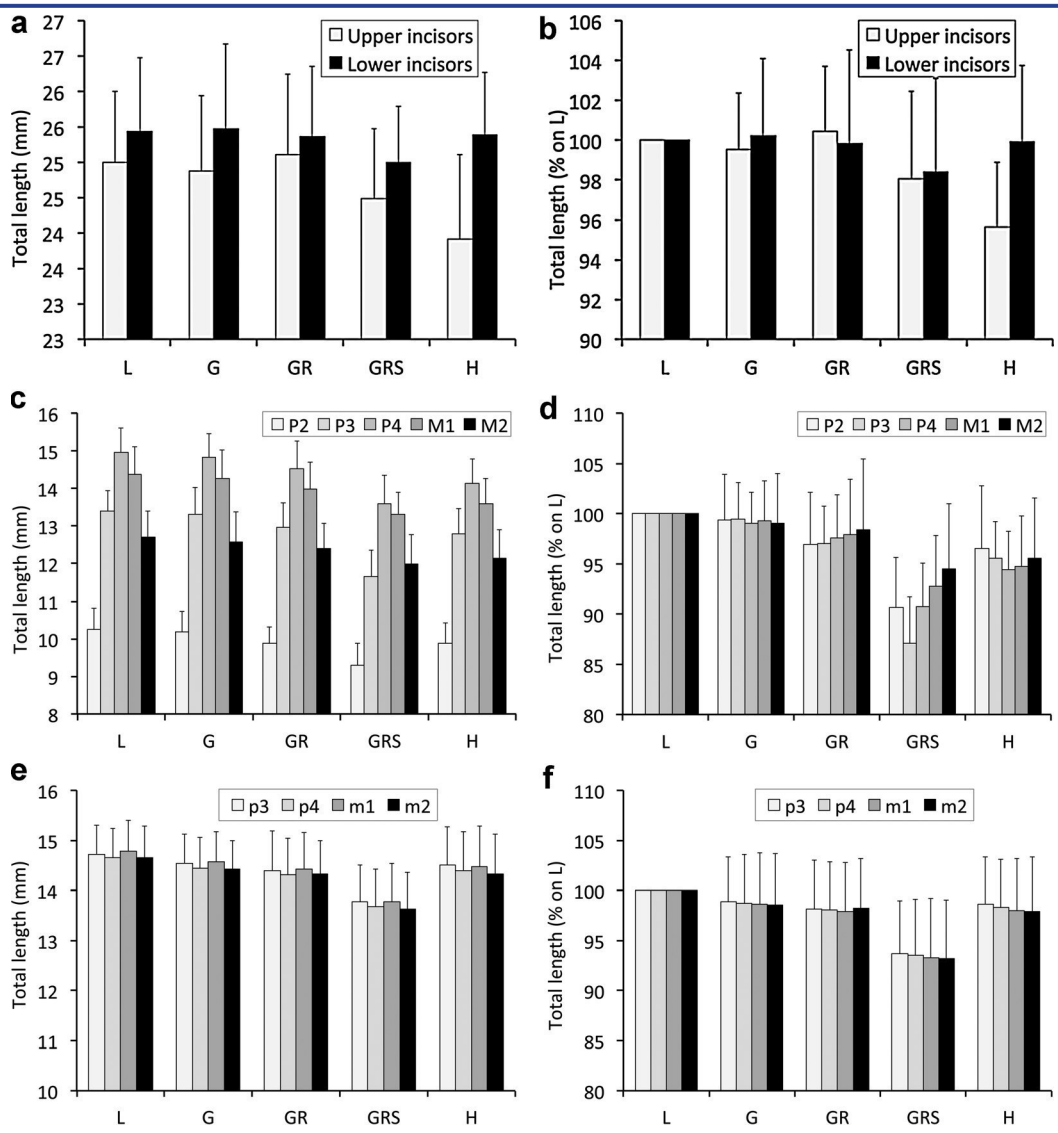

Figure 3. Mean total tooth lengths (averaged for right and left teeth) as measured on CT scans for (a,b) upper and lower incisors, (c,d) upper cheek teeth, and (e,f) lower cheek teeth, expressed as an absolute measure (in mm) in (a), (c), (e), and as a proportion of each individual's tooth on the lucerne diet in rabbits (*Oryctolagus cuniculus*) fed pelleted diets of lucerne (L), grass (G), grass and rice husks (GR), grass, rice husks and sand (GRS), and whole grass hay (H).

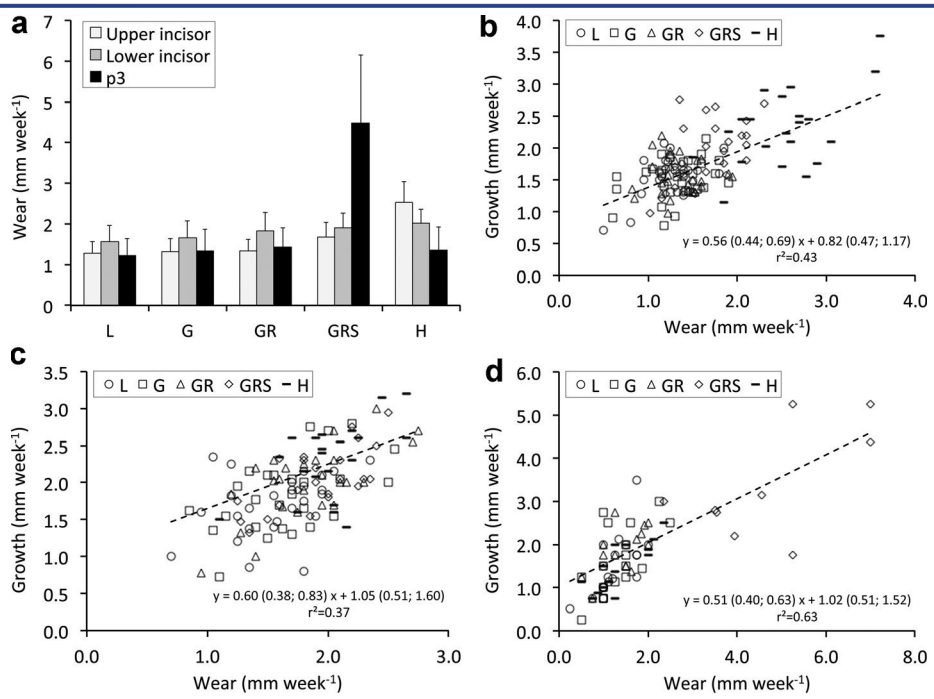

Figure 4. (a) Mean wear (in mm/week) of the upper and lower incisors and the lower first premolar (p3) in rabbits (*Oryctolagus cuniculus*) fed pelleted diets of lucerne (L), grass (G), grass and rice husks (GR), grass, rice husks and sand (GRS), and whole grass hay (H), and the relationship of wear and growth in (b) upper incisor, (c) lower incisor, (d) p3. Regression equations given with 95% confidence intervals of parameter estimates.

$P = 0.088$ for p3), and although there were significant diet × previous diet interactions in some cases ($F_{15,104} = 2.669$, $P < 0.01$ for I_1; $F_{15,72} = 2.827$, $P < 0.01$ for p3), these seldom revealed different effects within diets. Tooth position also had an influence on TL of cheek teeth, but only in the upper jaw ($F_{4,360} = 920.588$, $P < 0.0001$; lower jaw: $F_{3,297} = 1.473$, $P = 0.222$), with P4 having highest TL, followed by M1, P3, M2, and then P2 ($P < 0.0001$ in all cases). Actually, a significant diet × tooth interaction for upper cheek tooth TL ($F_{16,360} = 2.031$, $P = 0.011$) was found, indicating that tooth position influenced not only TL, but also mediated effects of diet on TL: for the GRS diet, TL was not significantly different from GR or H in P2 and M1 ($P = 0.092$–0.999), from GR in P3 ($P = 0.353$), from H in P4 ($P = 0.931$), and from G, GR, and H in M2 ($P = 0.451$–0.999).

Despite the fact that TL was not consistent across diets, measured tooth growth was positively and significantly related to tooth wear in I^1 ($b = 0.564$, $t_{111} = 8.796$, $P < 0.0001$; Fig. 4b), I_1 ($b = 0.603$, $t_{104} = 5.371$, $P < 0.0001$; Fig. 4c), and p3 ($b = 0.515$, $t_{57} = 8.833$, $P < 0.0001$; Fig. 4d). The 95% confidence intervals for the slopes of these relationships, even accounting for repeated measures across individuals in the models, were always less than 1.0. This indicates that compensatory growth was not exact (hypothesis 1), and hence diet effects on TL were evident (hypothesis 2). Actually, both growth (I^1: $F_{4,98} = 27.407$, $P < 0.0001$; p3: $F_{4,59} = 15.870$, $P < 0.0001$) and wear (I^1: $F_{4,103} = 67.109$, $P < 0.0001$; p3: $F_{4,50} = 53.322$, $P < 0.0001$) were influenced by diet in similar—but inverse—ways as compared with TL. That is, in I^1, growth and wear were highest for H, followed by GRS, and lowest for L, G, and GR (Bonferroni post hoc $P < 0.0001$ to < 0.01), whereas in p3, GRS was associated with higher values than all other diets ($P < 0.0001$ in all cases) (Fig. 4a). Differences in diet effects between incisors (most wear occurred on H diets) and p3 (most wear occurred on GRS diets) reflect predicted effects of gnawing (hypothesis 3a) versus chewing (hypothesis 3b) on the distribution of abrasive pressures throughout the mouth. Diet also had a significant effect on growth and wear of I_1 ($F_{4,110} = 9.824$, $P < 0.0001$; and $F_{4,93} = 9.730$, $P < 0.0001$, respectively), with H associated with fastest growth ($P < 0.0001$–0.010) and L and G diets associated with the least wear ($P < 0.0001$–0.038); wear on GR was significantly higher than that on L ($P = 0.011$). Interestingly, the time exposed to a particular diet influenced growth and wear in different ways, at least for p3: growth was faster in week 1 than week 2 on a particular diet ($F_{1,59} = 14.293$, $P < 0.001$), whereas wear was greater in week 2 ($F_{1,50} = 8.920$, $P = 0.004$).

Further evidence for differences in diet effects on wear in incisors versus cheek teeth was revealed by comparing relationships between wear and ADIAI (abrasiveness) or intake rate across

teeth. For I^1, both ADIAI and intake rate had significant positive effects on wear ($b = 0.024$, $t_{44} = 2.495$, $P = 0.016$; and $b = 0.024$, $t_{44} = 6.195$, $P < 0.0001$, respectively), whereas only ADIAI had a significant effect on p3 wear ($b = 0.110$, $t_{13} = 4.309$, $P < 0.001$; intake rate: $b = 0.005$, $t_{13} = 0.291$, $P = 0.776$). In I_1, however, relationships between wear with ADIAI and intake rate were never significant ($b = -0.006$–0.009; $P = 0.171$–0.983).

Differences in diet effects across tooth positions (diet × tooth interaction described above) indicate that a wear gradient may be evident along the cheek tooth row (hypothesis 3b). Analysis of TL data presented as a percentage of the value recorded for diet L (Fig. 3b,d,f) revealed a significant diet × tooth interaction (upper cheek teeth only: $F_{12,285} = 1.902$, $P = 0.034$; tooth effect for lower jaw: $F_{3,234} = 0.195$, $P = 0.900$) that is useful for understanding the hypothesized wear gradient. This interaction occurred because only on the most abrasive diet, GRS, did this relative TL measure differ significantly between teeth ($P = 0.195$–0.999 across teeth for all other diets), with P3 having lower values than M1 and M2 ($P < 0.001$–0.028).

Dental Abnormalities

TSP and TSF scores were significantly higher than zero for most teeth ($t_{79} = 2.237$–18.158, $P < 0.0001$–0.028) (Supplementary Materials). Only P2 and p3 had TSP scores not different from zero ($t_{79} = 1.000$, $P = 0.320$ and $t_{79} = 1.929$, $P = 0.057$, respectively). For TSF scores, only P2 did not differ from zero ($t_{79} = -1.000$, $P = 0.320$), whereas p4 never showed abnormalities in TSF and so the data had zero variance. These four cases were thus omitted from further analyses of dental abnormalities. For TA, however, the only teeth to present scores different from zero were P2 (<0, $t_{79} = -2.587$, $P = 0.012$) and p3 (>0 in the lower jaw, $t_{79} = 2.867$, $P < 0.01$) (Supplementary Materials). TA scores were not different from zero in P3 ($t_{79} = -1.348$, $P = 0.181$), P4 and M2 ($t_{79} = -1.000$, $P = 0.320$), and p4 ($t_{79} = 1.000$, $P = 0.320$), while M1, m1, and m2 never presented abnormalities. Hence, for TA scores, only data for P2 and p3 were retained in further analyses, and no diet × tooth interaction could be tested statistically for this measure.

Whereas TSP scores in the upper cheek teeth were only relevant on GRS, the lower cheek teeth had TSP scores on all diets (Supplementary Materials). Diet effects were significant for TSP scores (upper jaw $F_{4,297} = 13.759$, $P < 0.0001$, with GRS diets differing from all others, Bonferroni post hoc $P < 0.0001$; lower jaw $F_{4,218} = 3.981$, $P < 0.01$, with GR being higher than H, Bonferroni post hoc $P < 0.01$). There was no significant effect of tooth in the upper jaw ($F_{3,297} = 1.264$, $P = 0.287$), but in the lower jaw TSP scores were higher for m2 than p4 and m1 ($F_{2,218} = 3.981$, $P < 0.01$; Bonferroni post hoc $P < 0.0001$). The diet × tooth interaction term was never significant for TSP scores (upper jaw $F_{32,285} = 1.248$, $P = 0.293$; lower jaw $F_{8,210} = 1.417$, $P = 0.191$).

In contrast with TSP, TSF scores (Supplementary Materials) were not significantly affected by diet (upper jaw $F_{4,285} = 2.202$, $P = 0.069$; lower jaw $F_{4,210} = 1.175$, $P = 0.323$), but TSF scores did differ across teeth (upper jaw $F_{3,285} = 77.764$, $P < 0.0001$; lower jaw $F_{2,210} = 5.711$, $P < 0.01$) with both M2 and m2 having the highest scores ($P < 0.0001$–0.032). However, the tooth effect was not consistent across diets (diet × tooth interaction for upper jaw $F_{12,285} = 2.824$, $P < 0.01$; and lower jaw $F_{8,210} = 2.098$, $P = 0.037$). This occurred in the upper jaw because the difference between M1 with M2 was only significant for diets L, GR, and H ($P < 0.0001$–0.050), and on GRS there were in fact no differences across teeth ($P = 0.137$–0.999). Thus, diet did seem to influence TSF scores to some extent; whereas different teeth generally had different scores, the most abrasive diet (GRS) made all teeth similar. In the lower jaw, the difference between m2 with m1 and p3 was only significant for diet H ($P < 0.01$)—for all pelleted diets, teeth did not differ in TSF scores ($P = 0.305$–0.999).

TA scores (for which only P2 and p3 had scores different from zero, Supplementary Materials) differed across diets in both the upper ($F_{4,60} = 9.615$, $P < 0.0001$) and lower jaw ($F_{4,60} = 5.625$, $P < 0.001$), in that GRS diets were typically different from all other diets ($P < 0.0001$ to <0.01). TA scores for GRS were only not different from one other diet—GR, and then only in the lower jaw ($P = 0.252$).

Waviness—a proxy for overall abnormality in dentition—differed significantly across diets ($F_{4,60} = 5.863$, $P < 0.001$), with scores for GRS being higher than for all other diets (Bonferroni post hoc $P < 0.001$–0.012) (cf. Fig. 1b and Supplementary Materials).

DISCUSSION

This study confirms relevant effects of internal (dietary) and external abrasives on tooth wear, with important differences between the two functional tooth groups (incisors and cheek teeth), but also within the cheek tooth row. The observed patterns allow detailed interpretations on the process of tooth wear, including differences between maxillary and mandibular teeth. In our model species, tooth wear and growth were positively correlated, but correlation was not completely compensatory, which led to treatment-specific differences in TL. The growth rate for rabbit cheek teeth measured in this study was higher than previously reported in the literature.

Experimental Setup

There was an obvious change in body weight when feeding rabbits grass hay only. Most rabbits initially lost weight during this feeding period, although hay was provided ad libitum. In the second week of hay feeding, however, rabbits on average maintained their (lower) body mass (Fig. 1a). When fed pelleted diets, animals mostly gained weight. A dislike of the lucerne diet (L) was evident, which was already reported previously (Wolf et al., '99). In order to achieve isocaloric and isonitrogenous feeds, L had to comprise the highest proportion of lignocellulose (Table 1), which may have reduced their acceptance. Surprisingly,

the inclusion of sand did not affect the acceptance of diet GRS.

Hypothesis 1

Tooth growth compensates for wear; therefore, we expect tooth length to be relatively constant across diets and growth tightly correlated with wear.

We found a positive and significant relation between tooth growth and wear in upper incisors, lower incisors and p3, but compensatory growth was not exact, therefore resulting in diet-related different total TLs. In this study, effects of diet on teeth were monitored over constant periods of 2 weeks. We cannot address the question whether, over longer periods of time, growth would have compensated completely for wear, so that differences between diets would have disappeared (assuming an absolute length-set point). Alternatively, the detected differences in TL between diets in this study could have been an effect of an even shorter mismatch between growth and wear within a few initial days, after which the length measured in our study remains constant (assuming a relative growth/wear-setpoint). The finding that p3 growth was faster in week 1 than week 2, whereas wear was greater in week 2, could suggest that a stable equilibrium between wear and growth was not yet reached for the cheek teeth in the course of our experiment; however, this result could also reflect numerical differences in food intake between the weeks (Fig. 2b), with the second week representing a more or less stable equilibrium. The fact that for incisors, no difference in wear and growth between the weeks was evident, could represent evidence for a relative growth/wear-setpoint, indicating that absolute TL should vary (within boundaries) among rabbits on different diets. Fluctuations in incisor growth and wear over time in animals fed a constant diet documented by Wolf and Kamphues ('96), however, suggest that the balance between wear and growth represents a dynamic equilibrium.

Until now most authors assumed that growth and wear of cheek teeth are noticeably lower than that of incisors, and suggested a growth rate of 0.5–0.7 mm/week in cheek teeth (Table 2). The only exception were von Koenigswald and Golenishev ('79), who measured growth rates of cheek teeth by staining the growth-zone of the tooth with tetracycline and measured a growth rate in rabbit cheek teeth of 1.1–1.3 mm/week. In our study, growth rates of cheek teeth varied between 1.37–3.23 mm/week. These findings underline that growth of rabbit teeth is flexible, and that variation in response to wear should be expected.

Hypothesis 2

Small, detectable differences in wear between diets reflect diet abrasiveness and/or chewing activity.

Differences in TL, wear and growth can be linked to diet abrasiveness in this study for incisors and cheek teeth. We could not only confirm an effect of external abrasives, with TL mostly shorter/wear higher on diet GRS (containing sand) than on other diets, but also an effect of internal abrasives, where diets GR and H resulted in shorter cheek teeth than diets L and G, and wear on diet GR was higher in lower incisors than diet L. In the course of the debate whether internal abrasives (phytoliths) can actually cause relevant tooth wear and, correspondingly, drive evolutionary adaptations to this wear (see Introduction), our study thus provides evidence that internal abrasives do have a measurable effect on dental tissue. Differences in 3D surface texture between diets of different levels of internal abrasives (Schulz et al., 2013) thus will also translate into differences in wear. Whether the effects of internal abrasives can, in nature, ever act separately from the effect of external abrasives, remains debatable to date in particular in the context of grazing, with grasses containing high levels of phytoliths but also presumed to be particularly prone to grit contamination (Damuth and Janis, 2011). In ungulates, the finding that the interspecific correlation of hypsodonty with a habitat (i.e., external abrasives) proxy (precipitation) is not improved if mesowear (i.e., an internal abrasives proxy) is included in the analysis (Kaiser et al., 2013), could indicate that hypsodonty is mainly a reaction to external abrasives, the effects of internal abrasives notwithstanding. In primates, the finding that internal abrasives are related to enamel thickness (Rabenold and Pearson, 2011) might indicate that internal abrasives can also exert a selective pressure. For conclusive results, internal and external abrasives would have to be measured in the same forage samples to judge whether situations of high internal but low external abrasives (as suggested by Walker et al., '78) occur frequently.

Hypothesis 3

Functional differences between incisors and cheek teeth lead to different wear and growth on different diets.

a. *Incisors are worn more heavily when feeding whole hay that needs more gnawing as compared to pellets.*
b. *Cheek teeth, with a chewing action more independent from whether the diet is offered whole or pelleted, are worn more heavily with increasing dietary abrasiveness; external abrasives (sand) lead to a gradient in wear along the maxillary cheek tooth row whereas increased internal abrasives (phytoliths in rice hulls) do not lead to such a gradient (*Taylor et al., 2013*).*

This hypothesis was supported, as food intake rate (a proxy for chewing activity) was a significant factor for upper incisor wear, but not for cheek teeth or lower incisors. In incisors, differences in the chewing movement for whole forage and other feeds have been documented (Fig. 4 in Weijs and Dantuma, '81), with a prolonged posterior–anterior movement of the lower against the upper incisor on hay, which should increase both attrition and abrasion. In contrast, abrasives intake alone was correlated to wear of cheek teeth, indicating that chewing action of cheek teeth is more independent of whether the diet is offered whole or pelleted. This latter finding contradicts a hypothesis of Crossley (2003, Fig. 6), who suggested that mandibular grinding chewing

Table 2. Tooth growth and wear (in mm/week) in rabbits (*Oryctolagus cuniculus*) reported in the literature and from this study.

			Upper incisor		Lower incisor		Lower cheek teeth	
Study	Method	Diet	Growth	Wear	Growth	Wear	Growth	Wear
Shadle ('36)	Tooth mark	nm		2.0		2.4		
Spannbrucker et al. ('77)	nm	nm	2.1–2.3		2.1–2.3			
von Koenigswald and Golenishev ('79)	Enamel staining	nm	2.5		2.7		1.1–1.3	
Lobprise and Wiggs ('91)	nm	nm	2		2.4			
Wolf and Kamphues ('95)	Tooth mark	Carrots	1.68	1.61	1.64	1.45		
		Grass hay	1.74	1.65	1.82	1.61		
		Grain mix	1.39	1.21	1.25	1.14		
		Pellets	1.33	1.18	1.11	1.02		
Meredith (2007)	nm	nm	3	3	3	3	0.7	0.7
Harcourt-Brown (2009)	nm	nm	2.0–2.4		2.0–2.4			
Lord (2011)	nm	nm	2.0–2.5	2.0–2.5	2.0–2.5	2.0–2.5	0.6–0.7	
Schumacher (2011)	nm	nm					0.5–0.7	
Jekl and Redrobe (2013)	nm	nm	2–4	2–4	2–4	2–4	0.7–0.93	0.7–0.93
This study	Tooth mark[a]	L	1.54	1.27	1.72	1.57	1.47	1.23
		G	1.53	1.31	1.80	1.66	1.66	1.33
		GR	1.57	1.34	2.00	1.84	1.78	1.33
		GRS	1.98	1.68	2.09	1.90	3.23	4.47
		H	2.39	2.52	2.42	2.02	1.37	1.27

nm not mentioned; diets used in this study: complete pelleted diets (lucerne L, grass G, grass and rice hulls GR, grass and rice hulls and sands GRS) and grass hay (H).
[a]Incisors and the first lower premolar (p3) were marked.

movements are reduced in their extent on pelleted feeds as compared to natural vegetation (based on Weijs et al., '89, who did not report the composition of the pelleted diet they used), which should lead to less and more uneven wear of cheek teeth on pelleted diets. Weijs and Dantuma ('81) found that chewing muscle activity when chewing pellets did not differ significantly from that of chewing hay (but was lower for chewing carrots).

Corresponding to the different influence factors for the wear of incisors and cheek teeth, growth rates for incisors and cheek teeth were independent from each other, which requires a tooth-specific mechanism for adjusting growth to the tooth-specific wear. A gradient in the cheek tooth row developed particularly on GRS (external abrasives); a similar numerical effect of GR (internal abrasives only) (Fig. 3d) was not significant. Taylor et al. (2013) speculated that external abrasives might affect the front cheek teeth more, because they should be present on the outside of ingested plant material but become mixed into the ingesta bolus when passing further along the oral cavity towards the hind cheek teeth. This explanation appears inapplicable in our experimental setup, because the external abrasives (sand) were homogenously mixed into the pelleted diet GRS. The alternative explanation of Taylor et al. (2013) is that due to the geometry of mandibular movement, grinding movements should be more pronounced at tooth positions distant from the mandibular joint, that is, in the front cheek teeth, which might therefore experience higher wear. This explanation could also apply to our findings, although the fact that the P3, not the P2 was most heavily affected, remains unexplained. One could speculate on general differences in dental hardness, or on an effect of the diastema, which allows ingesta to evade the action of the P2 more by slipping off into the diastema than that in the case of the P3 where the preceding premolar prevents such "slipping."

The finding that for both incisors and cheek teeth, the most pronounced wear effect (triggered by chewing or abrasives, respectively) affects the maxillary teeth more than the mandibular ones might appear counter-intuitive, because the relative movement of teeth against each other (attrition) should not affect any position disproportionately. There is in fact ample evidence for ungulates that *lower* molars have more rounded cusps (more abraded attrition facets) than upper molars (Fortelius and Solounias, 2000; Franz-Odendaal and Kaiser, 2003; Kaiser and Fortelius, 2003), suggesting relatively more abrasion. Kaiser and Fortelius (2003) hypothesized that this difference might be simply due to the effect of gravity, but that explanation clearly does not fit the present results in rabbits. An ad hoc explanation of the relatively more pronounced abrasion of the *upper* teeth that we found in the rabbit experiment could be the following: ingesta rests, due to gravity, on the lower teeth, with which it is then moved against the upper dentition. The naturally occurring tooth spurs on the rabbits' lower cheek teeth (Supplementary Material) will

contribute to holding the ingesta in place. While remaining more stationary in relation to the lower teeth, the ingesta is then moved across the upper occlusal surface for grinding, resulting in relatively more ingesta movement along the upper than the lower occlusal surface during the chewing movement (Weijs and Dantuma, '81), which leads in turn to more abrasion affecting the maxillary teeth. In other words, we suggest, for the rabbit, a special case of the "inverted pestle-and-mortar" system (see Lucas, '79 for usage of this terminology), where the mandibular teeth represent a special kind of "pestle" both holding the food in place and grinding it along the "mortar" of the maxillary teeth. If this interpretation were correct, it would have to apply to the incisors, too, assuming that the relative movement of ingesta during their cutting action is also more pronounced in relation to the upper than to the lower teeth. Actually, anatomical drawings (Weijs and Dantuma, '81) and photographs of rabbit front teeth in Crossley (2003) indicate a concave occlusal surface of the maxiallary incisor, suggesting that a similar "inverted pestle-and-mortar" mechanism may be present in the incisors, too.

On the other hand, the observed wear patterns could be related to systemic differences between the upper and lower teeth themselves, such as a structural difference in the histology of dental tissues. A general explanation for why lower teeth should be more resistant to wear than upper teeth was in fact proposed by Fortelius ('85, p. 58f, Figs. 3 and 4) for ungulates: Because of the geometry of the occlusal facets, the direction of the main cutting edges of lower teeth is transversely to the enamel prisms, whereas on the upper teeth, their direction is along the prism axes, probably allowing relatively more tissue loss. Whether this explanation can be expanded to rabbits remains to be demonstrated. In rabbit cheek teeth, two forms of enamel have been described, a basal radial (more wear-resistant) and an outer irregular (less wear-resistant) enamel (von Koenigswald et al., 2010). Their respective distribution on upper and lower cheek teeth remains to be quantified, but in combination with the mentioned "inverted pestle-and-mortar" mechanism, the less-wear resistant irregular enamel might be particularly worn down on the mandibular cheek teeth. In the incisors, where the single-layered enamel structure is uniform between the upper and lower position (von Koenigswald, '96), differences in wear appear more likely to be due to other causes than enamel structure. More detailed knowledge on the relative movement of ingesta along dental surfaces, and about the histological directional anatomy of dental surfaces is required to test these hypotheses.

Hypothesis 4

Abnormal tooth wear will occur more frequently with excessive external abrasives (sand), and (according to 3b) affect the cheek teeth according to their position in the tooth row (anterior ones more affected).

We found significantly more dental abnormalities, including general tooth spurs (TSP), changes in the occlusal TSF, abnormal TAs and stepmouth formation, when feeding GRS pellets. This suggests that GRS not only increased general tooth wear but also the frequency of localized insults. However, in contrast to our prediction, the effects were not clearly more distinct in the anterior cheek teeth: whereas the anterior cheek teeth were affected for TA, this was not the case for TSP. Additionally, TSF abnormalities also affected the posterior cheek teeth. These findings give further evidence that abrasives (whether internal or external) did not change their position in the ingesta bolus in this study (the first hypothesis from Taylor et al., 2013 as explained above), and also do not correspond to presumed differences in tooth hardness, but suggest that differences in a tooth wear gradient due to external abrasives are rather linked to effects of differences in chewing movements between tooth positions (the second hypothesis from Taylor et al., 2013 as explained above).

Rabbits usually use their cheek teeth in a lateral figure-of-8 type pattern, which brings multiple cheek teeth in occlusion. It has been suggested than when feeding on pellets or grains, the vertical phase of the chewing action is more pronounced and the lateral one reduced, altering the pattern of tooth contact (Crossley, 2003; Lord, 2011). This could lead to dental abnormalities, because rabbits with abnormal chewing pattern do not efficiently wear down the whole occlusal surface. However, the results of this study do not support this hypothesis. Tooth spurs were not generally more frequent when feeding pellets than when feeding hay, and the wear of cheek teeth was not related to diet structure (see Hypothesis 2 above). While comprehensive tests for grain or pelleted feeds based on grain products remain to be performed (e.g., unpublished observations in Prebble and Meredith, 2014), our findings suggest that high-fiber pelleted feeds based on forages do not lead to more dental abnormalities in the short term than whole hay.

The ingestion of soil has been linked to gross dental abnormalities, such as stepmouth, in captive wild ruminants (Martin Jurado et al., 2008). Whether external abrasives cause such abnormalities or not will most likely depend on their particle size. At 0.233 mm (Table 1), the mean particle size of the sand used in this study was much larger than the dimensions usually given for phytoliths of 0.005–0.250 mm (reviewed in Strömberg, 2004). However, differences in the hypsodonty index and mesowear score in ungulates led Kaiser et al. (2013) to suggest that the grain size of the majority of external abrasives that affect herbivores must be at or even below the size of phytoliths. The effects of such external abrasives on teeth remain to be determined experimentally. It appears likely that such abrasives will not lead to dental abnormalities but only increase wear.

CONCLUSIONS

Our results underline that irrespective of the undoubted effects of external abrasives, internal abrasives have an effect on tooth wear and could therefore act as evolutionary drivers of dental adaptations; whether they do act in this manner will depend on

ecological scenarios where external abrasives do not represent an overriding signal. In veterinary practice, many clinicians believe in a constant tooth growth that needs to be worn down to prevent tooth elongation and malocclusion. We found that growth and wear of teeth vary, depending on intake of internal and external abrasives, and that wear and growth are tightly correlated. It is unclear how regulation between growth and wear takes place, and elucidating the associated feedback mechanisms represents a promising area of future research. Based on these findings, it is our opinion that diet alone may be less likely to cause dental problems in pet rabbits, due to flexible growth that reacts to wear. Other causes such as mineral imbalances or genetics should be considered when diagnosing dental disease, and a minerally balanced diet and breeding hygiene (not allowing affected animals to reproduce) may be the most promising prophylactic approaches. In cases where incisor overgrowth has occurred repeatedly, offering whole forages rather than pelleted diets appears the more promising strategy, but irrespective of differences in the absolute wear of the upper incisors, the use of forage-based pellets did not cause more abnormal wear than feeding whole hay in this study. Whether extreme diets, such as energy-dense concentrates of which small amounts meet energy requirements and that need not be chewed intensively (e.g., grain-based pellets) can have more deleterious effects due to an absence of wear than the diets of this study remains to be investigated in healthy animals.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Journal of Experimental Zoology A - Supplementary material

Growth and wear of incisor and cheek teeth in domestic rabbits *(Oryctolagus cuniculus)* fed diets of different abrasiveness

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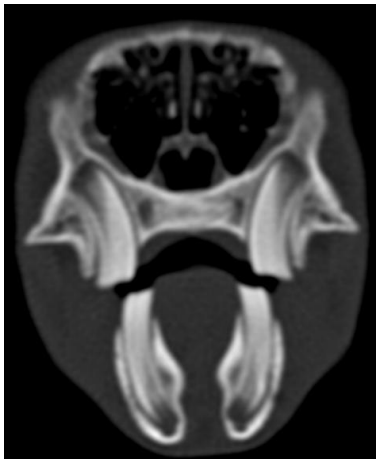
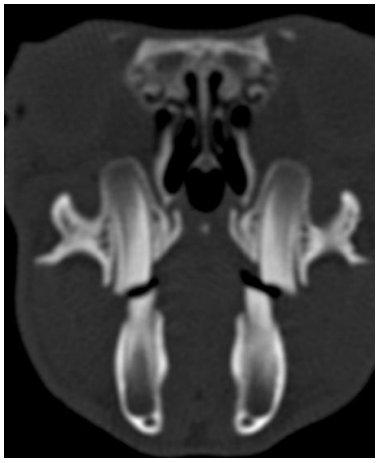
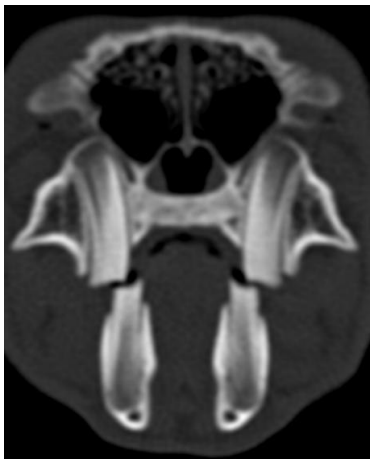
Scoring system: Tooth spurs (=TSP)



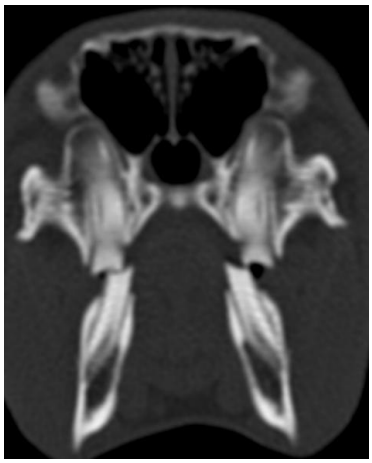
Score 0
No TSP on p3 and P3



Score 1
slightTSP on M1 on buccally

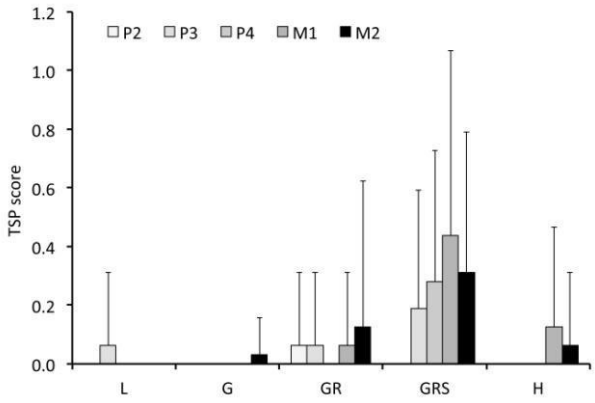


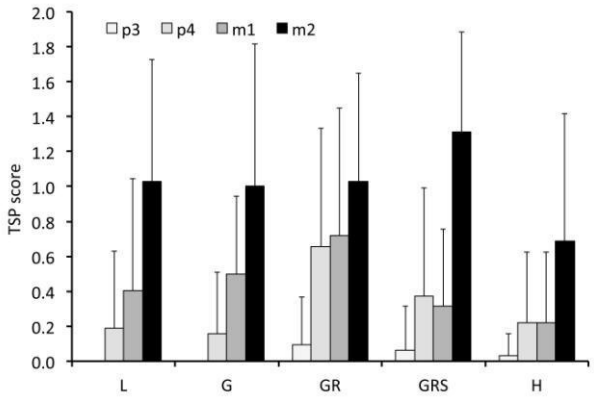
Score 2
Moderate TSP on p4lingually



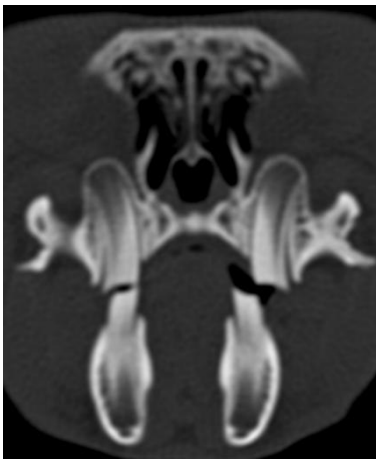
Score 3
Severe TSP on m1lingually

Results: TSP score on the different diets (for statistics, see main text)

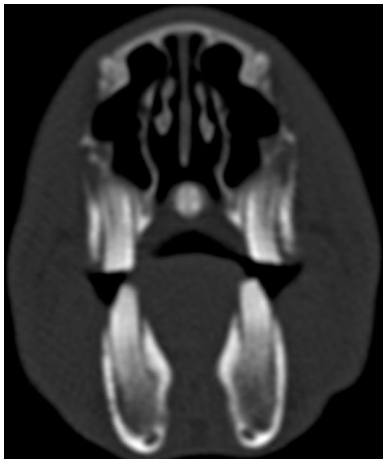




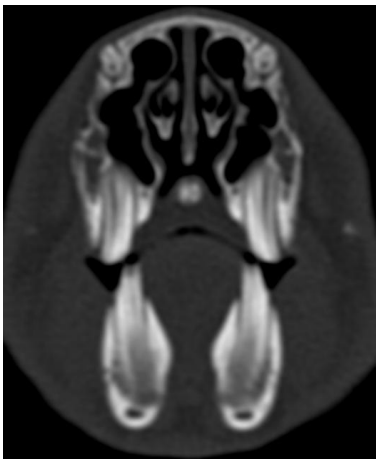
Scoring system: Tooth surface (=TSF)



Score 1
Concave TSF on M1

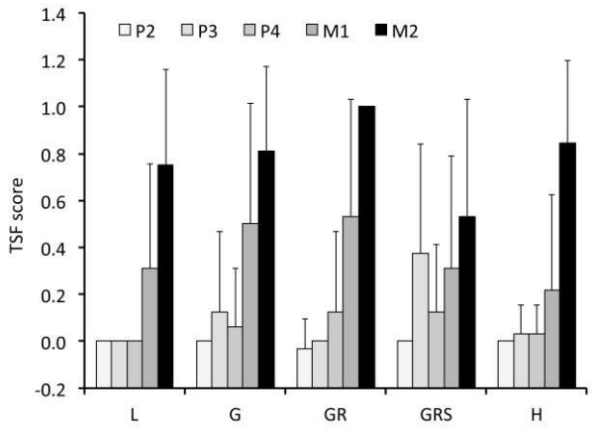


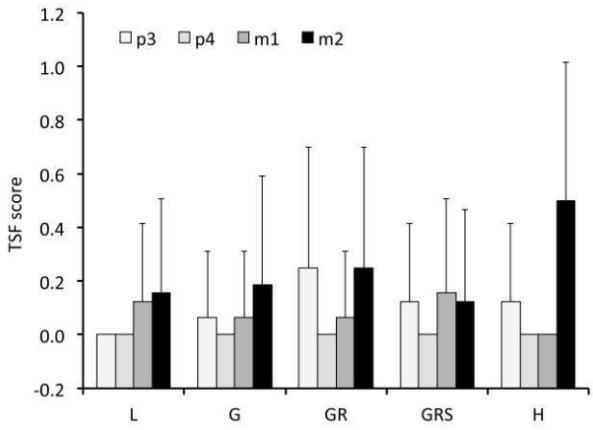
Score 0
Even TSF surface on P2



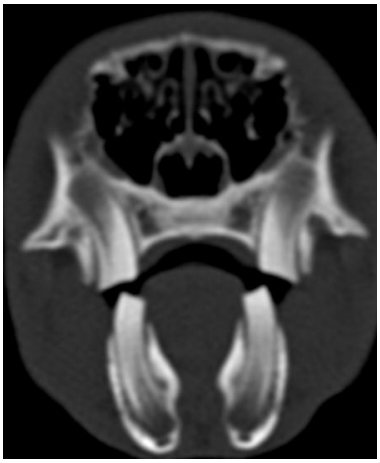
Score -1
Convex TSF surface on P2

Results: TSF score on the different diets (for statistics, see main text)

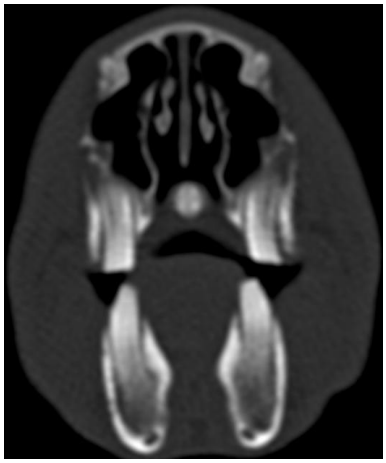




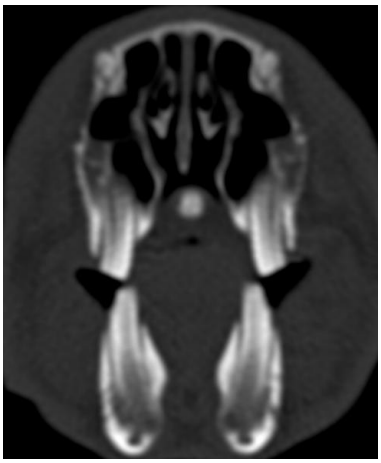
Scoring system: Tooth angle (=TA)



Score 1
TA sloped lingually on p3

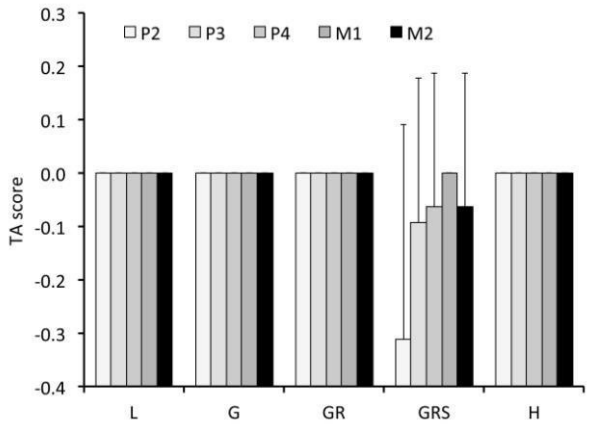


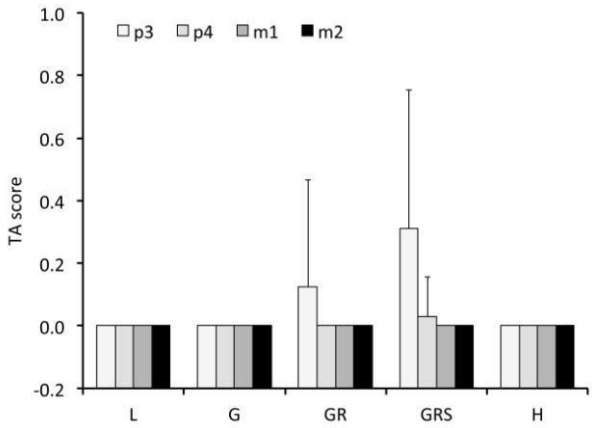
Score 0
Horizontal TA on P2



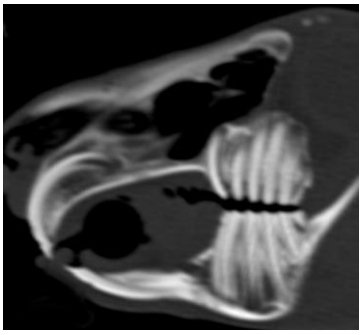
Score -1
TA sloped buccally on P2

Results: TA score on the different diets (for statistics, see main text)

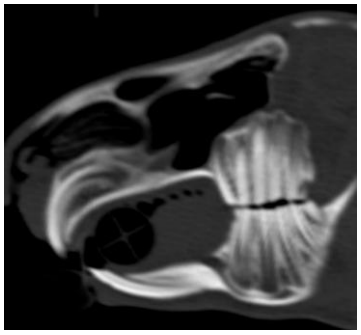




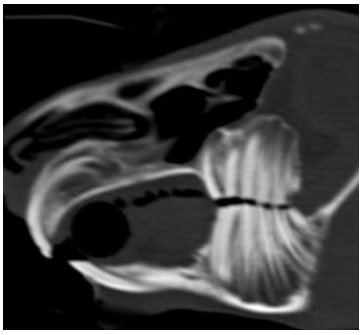
Scoring system: Stepmouth (= 'waviness' of the whole cheek dentition)



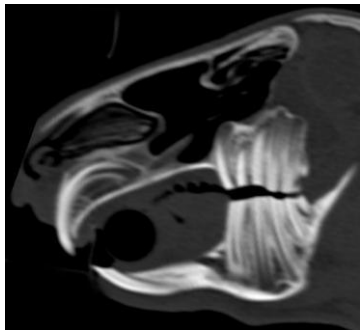
Score 0
normal dentition



Score 1
slight signs of waviness

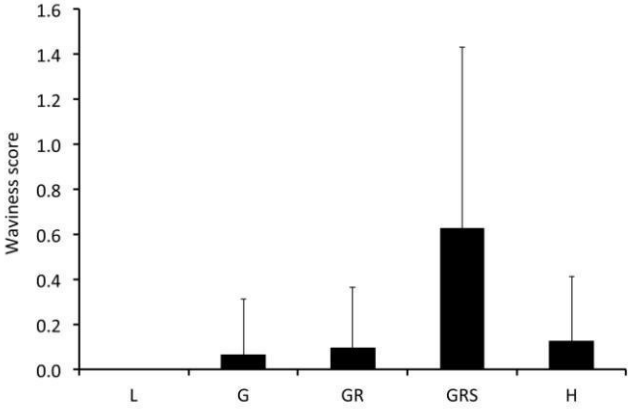


Score 2
moderate signs of waviness



Score 3
severe signs of waviness

Results: stepmouth score on the different diets (for statistics, see main text)



Tooth length and incisal wear and growth in guinea pigs (Cavia porcellus) fed diets of different abrasiveness

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Dental diseases are among the most important reasons for presenting guinea pigs (*Cavia porcellus*) and other rodents to veterinary clinics, but the etiopathology of this disease complex is unclear. Clinicians tend to believe that the ever-growing teeth of rabbits and rodents have a constant growth that needs to be worn down by the mastication of an appropriate diet. In this study we tested the effect of four different pelleted diets of increasing abrasiveness (due to both internal [phytoliths] and external abrasives [sand]) or whole grass hay fed for two weeks each in random order to 16 guinea pigs on incisor growth and wear, and tooth length of incisors and cheek teeth. There was a positive correlation between wear and growth of incisors. Tooth lengths depended both on internal and external abrasives, but only upper incisors were additionally affected by the feeding of whole hay. Diet effects were most prominent in anterior cheek teeth, in particular M1 and m1. Cheek tooth angle did not become shallower with decreasing diet abrasiveness, suggesting that a lack of dietary abrasiveness does not cause the typical 'bridge formation' of anterior cheek teeth frequently observed in guinea pigs. The findings suggest that other factors than diet abrasiveness, such as mineral imbalances and in particular hereditary malocclusion, are more likely causes for dental problems observed in this species.

Introduction

The teeth of extant caviomorph rodents are open rooted and continuously growing (hypselodont). This characteristic feature is an adaptation to ensure continuous tooth function in the presence of abrasion (Ungar 2010). In healthy animals, tooth growth ('eruption') and wear of dental tissue are well balanced, leading to a normal tooth shape and length. Tooth wear can be induced by either tooth-on-tooth contact (i.e. attrition) or by tooth-on-food contact (i.e. abrasion); food may contain internal abrasives, such as phytoliths, or external abrasives, like grit or dust (Butler 1972; Fortelius 1985; Kaiser et al. 2013).Any process interfering with either normal growth or wear of dental tissue will result in dental disease.

One of the most important disease complexes in guinea pigs and other rodents are dental abnormalities, and 12% (Langenecker et al. 2009) to 23.4% (Jekl et al. 2008) of guinea pigs presented in veterinary clinics are diagnosed with dental disease. Rodents suffering from dental disease show a variety of clinical signs like weight loss, chewing problems or a tendency to eat softer food, ptyalism (a condition formerly known as 'slobbers', Pollock 1951) followed by hair loss around the chin, poor fur quality and decreased grooming, facial swellings or abscesses, ocular discharge due

to obstruction or inflammation of the nasolacrimal duct, dysphagia, inability to carry out coprophagy and fewer or smaller fecal pellets(Verstraete 2003; Osofsky and Verstraete 2006; Harcourt-Brown 2007; Meredith 2007).

The etiology of this disease complex is still unclear and causes are frequently divided into congenital and acquired(Lennox 2008).Congenital causes are considered rare in rodents, but are often diagnosed in pet rabbits with maxillary brachygnathism (Capello 2004). Trauma can be one of the reasons for acquired dental disease. Traumatic injuries are a common presentation and are mostly due to falls or improper caging. Incisor fractures occur frequently; in this case the opposite incisor is not worn down efficiently and this may cause malocclusion(Capello 2004). Because guinea pigs are unable to synthesize vitamin C, a deficiency of this vitamin can lead to dental problems, because periodontal tissue and enamel formation is inadequate, which may lead to malocclusion(Boyle 1938).

The main presumed causes for acquired dental problems are either metabolic bone disease caused by dietary mineral imbalances, i.e. a calcium deficiency or husbandry conditions, or a lack of tooth wear due to an inadequate diet or insufficient gnawing opportunities. Harcourt-Brown (1995)suggests metabolic bone disease to be the cause for acquired dental disease in rabbits. The loss of supportive bone due to osteodystrophy would lead to loosening of the teeth and distorted growth. In degus (*Octodon degu)* a high-phosphorus diet with an improper calcium:phosphorus ratio was the cause for cheek tooth elongation, incisor depigmentation and enamel hypoplasia(Jekl et al. 2011a; Jekl et al. 2011b). Next to metabolic bone disease, an inadequate diet is thought to be one of the most important causes for insufficient tooth wear and malocclusion in rabbits and rodents (Crossley 2003; Meredith 2007; Harkness et al. 2010; Lord 2011). The widespread opinion among veterinarians is that feeding a diet rich in easily digestible carbohydrates but low in fiber will result in low overall food intake, and hence a low rate of dental attrition, insufficient dental wear and tooth elongation. It is therefore recommended to feed pet rabbits and rodents dried forages as a staple diet (e.g. Clauss 2012).

The surface of the rodent incisor teeth is covered with an enamel layer only on the labial, mesial and distal side but not on the lingual side, so gnawing leads to the characteristic chisel-shaped form of incisor teeth (Boehmer and Koestlin 1988; Wiggs and Lobprise 1990). Guinea pig cheek teeth have an occlusal plane that stands in a 30° angle, sloping down from lateral to medial(Capello 2004).Growth rates of guinea pig incisor teeth are given as 1.4-1.9 mm per week for upper incisors and 1.2-2.4mm per week for lower incisors(Shadle et al. 1938; Kamphues 2001; Table 2). It is a common belief among clinicians that hypselodont teeth show constant growth that needs to be matched by constant wear, induced by an appropriate diet, to avoid tooth elongation (Crossley 2000). In guinea pigs, a typical form of tooth elongation is lingual elongation of the mandibular premolars that may actually meet, build a typical ‘bridge’ and thus trap the tongue (Crossley 1995; Legendre 2002). During this malpositioning, the occlusal surface loses its buccal-to-medial slope but often becomes a nearly horizontal plane.

In a previous study, we demonstrated a significant and positive relationship between tooth growth and wear of incisor and cheek teeth in rabbits(Müller et al. 2014), dispelling the concept of constant growth rates but suggesting a feedback mechanism between tooth growth and wear that adapts to dietary influences. These findings were similar to previous findings where incisor growth and wear rates of rabbits, guinea pigs and other rodents varied on different diets (Wolf and Kamphues 1995; 1996). With respect to a current debate (Mainland 2003; Sanson et al. 2007; Damuth and Janis 2011; Lucas et al. 2013)whether herbivore teeth are only affected by external abrasives (dust, grit) or also by internal abrasives (phytoliths), we

found that rabbit teeth also experienced different levels of wear in relation to dietary internal abrasives concentrations (Müller et al. 2014).

To expand the study in rabbits, we aimed to test again the influences of varying levels of dietary abrasives in isocaloric diets and differences in food presentation (whole grass hay or pellets) on wear of incisor teeth and on tooth length in guinea pigs as a representative of rodent species. We tested four different, complete, pelleted diets based on lucerne (*Medicago sativa*), which naturally contains very low levels of internal abrasives (Wöhlbier 1983), grass, which contains higher levels of internal abrasives, and grass with the addition of rice hulls, which contain again higher levels (Wöhlbier 1983). The fourth pelleted diet included grass and rice hulls (internal abrasives), and additionally sand (external abrasives). To investigate the effect of a different diet form and therefore different chewing action, whole forage based on grass hay was tested as a fifth diet.

Based on our work in rabbits (Müller et al. 2014), the following hypotheses guided our approach:

1. Tooth growth and wear are positively correlated; nevertheless, differences in tooth length between diets, due to an incomplete compensation between growth and wear, can be detected.
2. Not only the diet with external abrasives (sand), but also diets with different levels of internal abrasives (phytoliths) have measureable effects on tooth wear and length.
3. Differences in wear and tooth length reflect diet abrasiveness, chewing action (whole forage *vs*. pelleted diets), and tooth position:
    a. due to functional differences between incisors and cheek teeth, incisors are more affected by the whole hay that requires gnawing, whereas cheek teeth are mainly affected by diet abrasiveness.
    b. in cheek teeth, a gradient occurs with anterior cheek teeth more distinctively affected by diet abrasiveness than posterior cheek teeth.
4. If ‘bridge formation’ of the cheek teeth is caused by diets of low abrasiveness, the tooth angle of the cheek teeth should be flatter on low-abrasion diets and steeper on high-abrasion diets. Again, this effect should be more distinct in anterior than posterior cheek teeth.

Materials and Methods

Animals and diets

This experiment was approved of by the Cantonal Veterinary Office in Zurich, Switzerland (No. 80/2012).The setup of the experiment was identical to the previous one in rabbits (Müller et al. 2014). Sixteen juvenile female guinea pigs(mean starting body mass 488±22 g; starting age 9-11 weeks) were kept individually in hutches (0.75 x 0.50m) on wood shavings and with plastic hides without other gnawing opportunities except their diet. Water was changed and supplemented daily with 200-400mg/l vitamin C (Redoxon®, Bayer AG, Zurich, Switzerland) (Quesenberry 1994)and was provided *ad libitum*. After a week of acclimatization the guinea pigs were randomly placed on one of five different diets for a two-week-period.

Diets consisted either of a grass hay fed as whole forage, or of complete pelleted diets formulated to be isocaloric and isonitrogenic, but of increasing abrasiveness from lucerne pellets (L), grass pellets (G), grass and rice hull pellets (GR), and grass and rice hull pellets with an addition of sand (GRS) (Table 1). Diets were fed ad libitum. Food intake was measured on a daily basis by weighing food offered and leftover. Guinea pigs were weighed at the end of each week, and the difference in body mass to the previous measure expressed as a percentage. After 14 days, a new randomly selected diet was introduced to each guinea pig with a two-day-interval of mixing the old and the new

diet to prevent digestive problems due to abrupt diet change. At the end of the experiment, all animals had received all diets.

To evaluate digestibility of the different diets, total faeces from each animal were collected and weighed for three consecutive days in the second week of every two-week-interval. Representative samples of feeds, leftovers (in the case of hay), and faeces were taken, dried at 60°C to constant weights, and prepared for further analyses by grinding. Protein, detergent fibres and acid detergent insoluble acid (ADIA) were measured using standard procedures (Van Soest et al. 1991; VDLUFA 2007; Hummel et al. 2011). A sample of the sand included in GRS was analyzed for mean particle size by wet sieving following to Fritz et al. (2012).

Dental measurements

In the dental nomenclature used here, I^1 and I_1 denote the upper and lower incisor, respectively, maxillary (upper) cheek teeth are denoted by capital letters (P or M for premolars and molars, respectively), mandibular (lower) cheek teeth by lower case letters (p or m), and the numbering of the cheek teeth represents their position in the tooth row (i.e., P4-M3 and p4-m3)(Thenius 1989). At the beginning of each two-week-interval, the guinea pigs' incisor teeth were burred and computed tomography (CT) of the head was carried out, and a final CT was taken at the end of the last feeding period. For these procedures the animals were placed under general anesthesia.

The animals were sedated with 0.5-1.5mg/kg midazolam (Dormicum®, Roche AG, Reinach, Switzerland) intramuscularly and anesthesia was induced and maintained with isoflurane administered in oxygen using an inhalation chamber20-30 minutes later. During anesthesia, punctual marks of the size of approximately 1mm were burred on the labial side of all four incisor teeth using a diamond burr (KaVo, EWL, Leutkirchi.A., Germany). Visible tooth crown length, and distances between gingival margin and mark (to assess tooth growth) and between mark and tooth edge (to asses tooth wear) of the four incisor teeth were measured(Wolf and Kamphues 1995) using an electronic digital caliper (Technocraft® Industrie, precision 0.01mm). The guinea pigs' recovery from anesthesia was uneventful, except in one case, where the animal suffered from aspiration pneumonia after aspirating saliva during anesthesia. The respective animal was successfully treated with 10mg/kg enrofloxacin (Baytril®, Bayer Health Care, Lyssach, Switzerland) subcutaneously for 7 days and meloxicam (Metacox®, Dr. E. Graeub AG, Bern, Switzerland) orally at a dose of initially 1mg/kg, and 0.5mg/kg for the following 4 days.

To monitor wear and growth of incisor teeth, caliper measurements were repeated a week later under manual restraint. All manual measurements were taken by the same examiner (JM).

Computed tomography and evaluation

CT scans (Fig. 1) were obtained using a 16 slice, spiral CT-scanner (Philips Brilliance 16, Philips Healthcare, Zurich, Switzerland). Images were acquired at 120KV, 117mA, a 10cm FOV with a slice thickness of 1mm. The guinea pig was positioned in ventral recumbency on the CT table to obtain transverse sections. The original data was reconstructed with a soft-tissue and a bone algorithm and was reviewed using a bone window setting (window width = 3814 HU, window level 594 HU) and a soft tissue window setting (window width = 270HU, window level = 100HU).CT images of the heads of all animals were investigated using OsiriX® software(PixmeoSarL, Bernex, Switzerland). Incisor teeth were measured in the sagittal plane, cheek teeth in the coronal plane with the help of an open polygon, a function that made it possible to measure curved structures. For each tooth the total length of the lingual and the buccal side was measured using this function as a curved structure from the base to the apex of the tooth. Additionally the 'tooth angle' (TA) of all cheek teeth was measured on the coronal plane. A line was drawn between the buccal edge of the occlusal surface of both cheek

teeth left and right as a reference line to measure tooth angle (see Supplementary material for detailed description). All CT scans were evaluated by the same examiner (JM) who was blinded to the diet that the respective scans represented.

Statistics

Data were analyzed using mixed-effects linear models (MELMs), accounting for repeated measurements from the same individuals by including Individual as a random effect to ensure correct error terms were being compared. Initially, we compared the general nutritional status of guinea pigs across diet treatments, including Diet as a main effect, and evaluating several response variables: body mass at the end of the relevant trial period; the relative (as a percentage of initial body mass) change in body mass over the relevant trial period (body $mass_{change(\%)}$); dry matter intake (DMI); acid detergent insoluble ash intake (ADIAI); and dry matter digestibility (DMD). Except for DMD (which was only measured in the second week of each diet regime), we also included the Week on a particular diet treatment (1 or 2), and the Previous diet treatment (body mass variables only), as well as interactions between these terms with Diet, to account for autocorrelation and other temporal effects. We then assessed diet effects on tooth length (TL), growth, and wear using similar MELMs, except that for TL (week 2 only) we also included Tooth as a main effect (as well as its interaction with Diet) to test for differences along the cheek tooth row. We also analyzed wear and growth responses to ADIAI independently of the specific diet treatment, by replacing Diet with continuous quantitative variables - i.e. wear as an effect on growth, and ADIAI (abrasiveness of diet) as effects on wear. Finally, to check for diet effects and tooth row position on the slope of the occlusal surface (hypothesis 4), we used MELMs with the tooth angle(TA)as dependent variable, and Diet and Tooth as main and interactive effects.

Data for incisors and cheek teeth, and for upper and lower teeth, were analyzed in separate MELMs. In cases where interaction terms were not significant (P>0.05), these were removed and the relevant models repeated without them. Bonferroni*post hoc* tests were used for multiple comparisons where necessary. All analyses were carried out in STATISTICA version 8(Statsoft_Inc 2007).

Results

General diet effects

Diet had a negligible influence on guinea pig body mass. Although significant ($F_{4,119}$=2.700, P=0.034), diet only had an effect on body mass because of a higher mean for diet GRS than G (Bonferonni*post hoc* P=0.018; all other multiple comparisons P=0.290 to 1.000). For % body mass change, there was no effect of diet ($F_{4,119}$=1.266, P=0.303). The previous diet also had no effect on either body mass variable ($F_{4,119}$=0.560, P=0.692; $F_{4,119}$=1.086, P=0.367, respectively). Body mass also did not change between weeks 1 and 2 on a diet treatment ($F_{1,119}$=0.880, P=0.350; $F_{1,119}$=0.005, P=0.946, respectively; Fig. 2a).

The DMI, ADIAI, and DMD of guinea pigs differed across diet regimes ($F_{4,104}$=35.470, P<0.0001;$F_{4,104}$=1541.92, P<0.0001;$F_{4,60}$=15.053, P<0.0001, respectively). DMI was higher for G than all other diets (P<0.0001), followed by L and GR (P=0.346), which in turn had higher DMI than GRS and H (P<0.0001 to 0.050). Guinea pigs increased DMI from week 1 to week 2 of particular diet treatments ($F_{1,104}$=40.810, P<0.0001; $F_{1,104}$=19.030, P<0.0001, respectively; Fig. 2b).ADIAI differed significantly for all diets (P<0.0001), with GRS>H>GR>G>L (Fig. 2c). DMD for H was higher than the other diets (P<0.0001 to 0.030), except for GRS (P=0.235), whereas for G DMD was lower than other diets (P<0.0001 to <0.001), except for L (P=0.383). Diets L, GR, and GRS all had similarly intermediate DMD values (P=0.075 to 1.000).

Effects on tooth length, growth and wear

Diet had a significant influence on TL of I^1($F_{4,60}$=5.620, P<0.001), but not I_1($F_{4,60}$=0.450, P=0.770). The former occurred because TL for $I^1$was lower on H than for other diets (Fig. 3a; P<0.0001 to 0.017).This result is more evident when TL for each diet was expressed relative to data for diet L (Fig. 3b; $F_{4,60}$=5.520, P<0.001 and $F_{4,60}$=0.410, P=0.803 for $I^1$and I_1, respectively). TL of incisors for all other diets besides H never differed (P=1.000 for absolute TL and TL expressed relative to L).

Diet also had a significant effect on TL of upper (Fig. 3c;$F_{4,285}$=20.700, P<0.0001) but not lower cheek teeth (Fig. 3e;$F_{4,297}$=0.900, P=0.439). When expressed as a percentage relative to TL for the L diet, however, the diet effect was significant in both the upper (Fig. 3d; $F_{4,297}$=4.440, P<0.01) and lower (Fig. 3f; $F_{4,297}$=4.800, P<0.001) tooth rows. In contrast with incisors, for which TL differed on H compared with other diets, in cheek teeth GRS was generally associated with lower TL than other diets (P<0.0001 for upper cheek teeth[but also, GR>G, P=0.034]; P<0.01 for lower cheek teeth, except here TL for GRS and GR were similar [P=0.441]). In both tooth rows, there were also significant changes across cheek teeth in TL ($F_{3,285}$=1034.600, P<0.0001; $F_{3,285}$=496.400, P<0.0001, for upper and lower rows, respectively; Fig. 3c & e), and in TL expressed relative to L ($F_{3,297}$=8.800, P<0.0001; $F_{3,297}$=5.900, P<0.001, for upper and lower rows, respectively; Fig. 3d & f).In the upper tooth row, TL was highest for P4, followed by M1, M2, and M3 (P<0.0001).The reverse pattern was observed in the lower tooth row (P<0.0001, except that TL for m1 and m2 were similar [P=1.000]);generally, TL was highest for m3, followed by m2, m1, and p4. A significant diet*tooth interaction was found for TL of the upper cheek tooth row ($F_{12,285}$=2.100, P=0.014), because the pattern of lower TL for GRS than other diets persisted, except that GRS did not differ from L in M1 and M2 (P=1.000), from G for M3 (P=1.000), from GR in P4(P=0.170), M1 and M3 (P=1.000), and from H in P4 (P=0.215).

Wear and growth measured manually in incisors also differed significantly across diets (I^1: $F_{4,104}$=44.662, P<0.0001; $F_{4,113}$=6.132, P<0.001, respectively; I_1: $F_{4,113}$=4.593, P<0.01; $F_{4,114}$=4.370, P<0.01).I^1wear was higher for H than other diets (P<0.0001), but in I_1 GRS differed from other diets in having higher wear (Fig. 4a; P<0.01 to 0.030[except for GR,P=1.000]).In both cases, growth was higher for H than most other diets (Fig. 4b; P<0.001 to 0.013).In addition to this, animal's previous diets had an effect on incisal wear and growth that was either significant overall (I_1wear: $F_{4,113}$=1.256, P<0.01), or at least approaching significance (I^1wear $F_{4,101}$=2.299, P=0.064; I^1growth: $F_{4,113}$=2.078, P=0.088), with the exception of I_1growth ($F_{4,114}$=1.237, P=0.299). Multiple comparisons showed that animals switched from H to other diets generally experienced more incisal wear (Fig. 4c; I_1: P=0.015 to 0.029, except for GRS where P=0.178; I^1H>G P=0.019, but H vs. all other diets was similar [P=0.170 to 1.000]) and I^1growth (Fig. 4d; P<0.001 to <0.01, except for H vs. GRS where P=0.546).

Incisal wear and growth typically did not differ between weeks 1 and 2 of a particular diet regime (P=0.605 to 0.940), except for I_1growth (P<0.01). However, a significant diet*week interaction occurred in all cases (I^1wear$F_{4,101}$=3.243, P=0.015; I_1wear$F_{4,113}$=3.262, P=0.014; I^1growth $F_{4,113}$=3.656, P<0.01; I_1growth $F_{4,114}$=3.549, P<0.01). For I^1wear, higher wear for H than other diets occurred during both weeks; evidently, the diet*week interaction did not influence the general pattern. However, in I_1, the difference in wear between GRS and other diets never occurred within the same week, i.e. differences occurred across weeks, but not within either week 1 or week 2. The only exception is that GRS and H differed after 1 week feeding on each (P<0.01). Thus, the finding that I_1 wear differed between GRS and other diets may be considered a

weak signal. ForI^1, growth was higher for H than other diets only after 2 weeks (P=1.000 for week 1), but differed from GRS only during week 1 of a trial period (P=1.000 for week 2). In I_1, differences in growth across diets only ever occurred after 2 weeks (P=1.000 for all cases for week 1).

Despite that TL was not consistent across diets, measured tooth growth was positively and significantly related to tooth wear in I^1 (Fig. 4e;b=0.25, t_{113}=3.610, P<0.001) and I_1(Fig. 4f;b=0.19, t_{125}=2.502, P=0.014). The 95% confidence intervals for the slopes of these relationships, even accounting for repeated measures across individuals in the models, were always less than 1.0, indicating that compensatory growth was not exact, and hence diet effects on TL should be expected. Dietary abrasiveness (ADIAI) had a significant, albeit weak, effect on wear of I_1 (b=0.078, t_{113}=3.218, P<0.01), but did not influence wear of I^1significantly (b=0.044, t_{125}=1.533, P=0.128).

Effects on tooth shape

TA of both upper and lower cheek teeth differed significantly across diets ($F_{4,285}$=16.420, P<0.0001; $F_{4,285}$=2.530, P=0.041, respectively) and across teeth ($F_{3,285}$=17.600, P<0.0001; $F_{3,285}$=38.350, P<0.0001, respectively).In both cases, however, there was a significant diet*tooth interaction ($F_{12,285}$=4.180, P<0.0001; $F_{12,285}$=3.330, P<0.001, respectively). This meant that in the upper tooth row, the only significant differences in TA occurred because low values were recorded for GRS relative to other diets, but only for P4 and M1 (P<0.0001 to 0.042; Fig. 5a). In the lower tooth row, GRS also had lower TA values than other diets, but only for m1 (P<0.0001 to 0.015; Fig. 5b). In addition, TA values were lower for p4 than m3 on all diets (P<0.0001 to 0.033), except for L (P=0.128).

Discussion

The tooth wear patterns documented in this study corroborate previous findings in rabbits (Müller et al. 2014), and thus indicate that these patterns are not species-specific but probably valid in a larger variety of species, and represent general aspects of mammalian tooth wear. In discussing the results, we will refer to the rabbit study without indicating the reference every time.

Experimental setup

There was no relevant influence of diet on body mass and no change in body mass was seen between week 1 and 2 of each feeding period. All animals gained body mass throughout the study period, because they were still growing to maturity. The only exception was a higher body mass on diet GRS that might have been due to sand accumulation in the digestive tract (without evident clinical consequences). In comparison to rabbits, guinea pigs could maintain and even increase their body mass when feeding on hay only, indicating a higher capacity to digest roughages that has been documented in digestion studies (e.g. Franz et al. 2011). Correspondingly, while DMD was similar between the species for diets L and G, guinea pigs achieved higher DMD for GR, GRS and in particular H (56 % vs. 45 % in rabbits). In the guinea pigs, DMD and DMI appeared to be linked, with higher DMI associated with lower DMD, most likely due to a decrease in digesta retention at higher DMI as observed in other species (e.g. Clauss et al. 2014). In contrast to rabbits, DMI increased from week 1 to week 2, suggesting that guinea pigs required more time than rabbits to get accustomed to a new diet. The guinea pigs seemed to prefer diet G, whereas rabbits ingested similar amounts of G, GR or GRS. Nevertheless, as in rabbits, there was no evident particular intake reduction on GRS, indicating that the presence of sand in the diet was also tolerated. ADIAI increased in guinea pigs from L<G<GR<H<GRS as planned. Experimental work with guinea pigs was more limited than in rabbits, because it was not possible to use a burr mark on the cheek teeth, and because intake rates could not be quantified due to the general shyness of our experimental animals that would not eat in the presence of an observer.

Hypothesis 1: Tooth growth and wear are positively correlated; nevertheless, differences in tooth length between diets, due to an incomplete compensation between growth and wear, can be detected.

As in rabbits, we found a positive relationship between tooth growth and wear of upper and lower incisors in guinea pigs, thus confirming other studies (reviewed in Müller et al. 2014) that indicate that in ever-growing teeth, the growth rate is regulated to compensate for wear. Evidently, husbandry concepts based on the assumption of a constant dental growth rate that needs to be met by sufficient wear to avoid tooth overgrowth cannot be sustained in the face of these results, except for diets of very low abrasiveness that additionally do not stimulate gnawing: extrapolating the regression lines in Fig. 4e to conditions of no wear, a constant growth of the upper incisor results (but not for the lower incisor in Fig. 4f). Probably, such diets can be considered rare. In the short time course of this study, feeding pelleted diets instead of hay did not lead to indications of abnormal tooth wear. Whether more energy-dense pelleted diets or grain mixes can lead to such abnormalities awaits further investigation. Therefore, direct dietary effect on tooth wear appears less likely to be a cause for the frequently observed dental problems, shifting the focus on other factors that influence occlusion – such as mineral imbalances or genetic predisposition. Clearly, the concept to reduce dental abnormalities in pet rodents by avoiding breeding with affected animals requires further investigation.

The feedback mechanism that adapts growth to wear in rabbits and rodents has not been identified to date. Differences between upper and lower incisors in this study, additional differences between incisors and cheek teeth in the rabbit study (Müller et al. 2014), as well as observations on individual, broken-off incisors that have a faster growth rate than their contralateral neighbour until back in occlusion (Schour and Medak 1951; Ness 1956) suggest that such a feedback mechanism must operate on the level of the individual tooth. Occlusal contact has been identified as the major determinant of rodent dental eruption in a series of experiments using the incisors of rats (*Rattus norwegicus*) as the model teeth (Michaeli et al. 1974). In contrast to the rabbits, the previous week's diet had significant effects on incisal wear and growth in the guinea pigs, opening the possibility that the feedback mechanism might have a certain reaction delay. Whether this feedback mechanism has fixed 'setpoints' would have to be investigated by longer-term studies; if such setpoints existed, one would expect no difference in tooth length between diets after a sufficiently long period of time (evidently, longer than the two weeks of our individual feeding regimes). Alternatively, the feedback mechanism might operate with a relative setpoint that allows for a range of tooth lengths (and consequently also differences in the range of movement in the mandibular joint). Fluctuations in incisor growth and wear over time in animals fed a constant diet documented by Wolf and Kamphues (1996) suggest that this latter option is more likely to be the case.

Hypothesis 2: Not only the diet with external abrasives (sand), but also diets with different levels of internal abrasives (phytoliths) have measureable effects on tooth wear and length

In the current debate on the main triggers of dental wear in mammals, it has been suggested that phytoliths cannot wear down tooth enamel (Sanson et al. 2007) or can only cause limited damage (Lucas et al. 2013). In contrast, micro- and macro-anatomical results in rabbits (Schulz et al. 2013; Müller et al. 2014), as well as the intermediate position in tooth length and wear for diet GR in the guinea pigs indicates that internal abrasives actually do wear down teeth. Therefore, irrespective of a strong influence of external abrasives on tooth wear (Damuth and Janis 2011; Kaiser et al. 2013), assumptions that variation in the internal abrasives content of natural diets can trigger evolutionary adaptations (Rabenold and Pearson 2011) appear reasonable.

Hypothesis 3a: Incisors are more affected by the whole hay that requires gnawing, whereas cheek teeth are mainly affected by diet abrasiveness.

Diet had a significant effect on tooth length of upper incisors and upper and lower cheek teeth in this study. The effect on the lower cheek teeth, although numerically present (Fig. 3e), could only be demonstrated statistically when expressed relative to tooth length on the diet with the lowest level of abrasives (Fig. 3f). The length of the lower incisors was unaffected by diet, but dietary effects could be shown on wear and growth also in these teeth. As in rabbits, the length of the $I^1$was shorter, and wear was higher, on diet H, whereas wear of $I_1$was higher on diet GRS. And as in rabbits, upper cheek tooth length was most reduced on GRS, and abrasives appeared to affect upper cheek teeth to a greater extent than lower cheek teeth. These similarities suggest a similar mode of action in rabbits and guinea pigs both during incisal gnawing and chewing(reviewed in Müller et al. 2014), and suggest that feeding whole forages, while important when increased wear of incisors is aimed at, will not differ in the wear effect on cheek teeth when compared to high-fibre pellets.

Hypothesis 3b: In cheek teeth, a gradient occurs with anterior cheek teeth more distinctively affected by diet abrasiveness than posterior cheek teeth.

In rabbits(Müller et al. 2014), but also in rhinoceroses (Taylor et al. 2013), anterior cheek teeth experience more wear than posterior ones, with a resulting tooth wear gradient. Additionally, dental abnormalities also affect these cheek teeth positions more frequently in rabbits, guinea pigs and chinchillas (Jekl et al. 2008). These observations are again corroborated by the findings of this study. This pattern begs for a functional explanation. Taylor et al. (2013)suggested that due to the geometry of mandibular movement, grinding movements should be more pronounced at tooth positions distant from the mandibular joint, cranial cheek teeth might thus experience more pronounced wear, and might therefore also be more predisposed to abnormal tooth wear.Müller et al. (2014)additionally speculated that this effect might be most pronounced for the second tooth in the row, because ingesta cannot easily evade this position, whereas it could slip off into the diastema from the first tooth of the cheek row. Alternatively, systematic differences in dental anatomy or material characteristics of the tooth tissues cannot be ruled out. Further studies, including for example fluoroscopic observations on the behaviour of radiodense abrasives during mastication, are required to find more conclusive answers.

Hypothesis 4: Cheek tooth angle will become steeper with increasing dietary abrasiveness

The rationale of this hypothesis was that in guinea pigs, a common dental abnormality that is also linked to diets of poor abrasiveness is ‘bridge formation’ of the anterior cheek teeth (Crossley 1995; Legendre 2002), a process during which the corresponding teeth necessarily lose their steep angulation of the occlusal surface, which becomes horizontal. If this condition was really linked to dietary abrasiveness, and if it could be prevented by diets of increased abrasiveness, then steeper angles would be expected on diets of higher abrasiveness. Usually a tooth angle of about 30°is considered normal in healthy guinea pigs(Capello 2004; Reiter 2008), but our results suggest systematic differences along the tooth row, with an increase in steepness from M1 to M3 and m1 to m3 (Fig. 5).

Tooth angle differed significantly across diets, but in the opposite direction from the predicted effect. Although we cannot exclude different findings on diets of very low abrasiveness that require very little chewing, this result is a further indication that diet abrasiveness plays a minor role in dental abnormalities observed in this species. Again, this shifts the focus to other factors, in particular hereditary ones. Although detailed studies are lacking, Rest et al. (1982) reported the occurrence of malocclusion, including photographic documentation of

'bridge formation' of the mandibular cheek teeth, in a colony of guinea pigs where the problem could be reduced when excluding relatives of affected animals from breeding.

As with overall wear, the anterior cheek teeth, and in particular M1 and m1, were most affected by external abrasives. These findings support the reflections on cheek tooth position elaborated above. In this study, the change in tooth angle in the anterior cheek teeth evoked by external abrasives might have affected occlusal integrity (cf. Fig. 1), supporting the finding in rabbits that high loads of coarse-grained external abrasives lead to abnormal tooth shape patterns (Müller et al. 2014).

Conclusions

In general, we found very similar effects of dietary abrasiveness in guinea pigs as in a previous study in rabbits. Tooth growth and wear are positively correlated, and depend on the intake of external and internal abrasives. Feedback mechanisms operating on the level of individual teeth control tooth length. Given that occlusal contact is the major determinant of rodent dental eruption (Michaeli et al. 1974), dietary abrasiveness (or lack thereof) appears a poor candidate for the explanation of dental abnormalities including elongation.

A common dental anomaly in guinea pigs is tooth elongation of mandibular cheek teeth with 'bridge formation' (Crossley 1995), and diminished lateral excursion of the mandible has been suggested as a possible causative factor(Legendre 2002). The only measurements of such chewing movements, reported by Byrd (1981),however do not suggest a systematic difference in the lateral chewing movement between carrots and a pelleted food. Although we cannot make conclusions for diets of lower abrasiveness that may be chewed less intensively than the pelleted diets of this study (which would all be considered 'high-fibre' when compared to pelleted diets commonly in use for rabbits and guinea pigs; Kamphues et al. 2009), the feeding of pelleted diets per se does not appear to induce cheek teeth abnormalities. Diet may not be as important a factor in causing dental disease as often perceived. Rather, lack of occlusion, either due to acquired or hereditary factors, appears more plausible, and both effects of minerally imablanced diets (Jekl and Redrobe 2013)and genetic involvement (Rest et al. 1982) have been demonstrated in rodents. Therefore, we recommend that clinicians take into account these other reasons for dental disease, such as mineral imbalances (i.e. metabolic bone disease) due to inappropriate husbandry or selective feeding when receiving mixed diets ad libitum (Harcourt-Brown 1996; Prebble and Meredith 2014), and we advise to prevent affected animals from breeding.

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Table 1. Composition of different complete pelleted diets (lucerne L, grass G, grass & rice hulls GR, grass & rice hulls & sands GRS) and grass hay (H) fed to guinea pigs (*Cavia porcellus*)

	L	G	GR	GRS	H
Ingredients					
Lucerne meal (%)	60.0	-	-	-	-
Grass meal (%)	-	60.0	64.8	64.8	-
Rice hulls (%)	-	-	20.0	20.0	-
Sand[1] (%)	-	-	-	5.0	-
Pure lignocellulose (%)	33.8	27.4	5.0	-	-
Soybean meal (%)	-	7.0	5.0	5.0	-
Molasses (%)	3.0	3.0	3.0	3.0	-
Lignobond (%)	2.0	2.0	2.0	2.0	-
Soy oil (%)	1.0	0.4	-	-	-
Mineral/Vitamin premix (%)	0.2	0.2	0.2	0.2	-
Dry matter (% as fed)	91.4	91.9	91.8	92.2	90.8
Nutrient composition (g/kg DM)					
Total ash	79	64	75	130	104
Crude protein	102	90	97	85	109
aNDFom[2]	578	600	487	459	579
ADFom[3]	434	403	322	299	354
ADL[4]	131	110	74	65	52
ADIA[5]	05	16	24	77	38
Dry matter digestibility (%)	39.7±9.3	34.3±8.1	41.2±5.7	40.7±11.1	45.1±4.1

[1]Sand for Playgrounds, grainsize 0-1mm, REDSUN garden products B.V., Heijen, Denmark, mean particle size measured by sieve analyses as dMEAN(Fritz et al. 2012)of 0.233mm
[2]aNDFom neutral detergent fibre, determined using amylase and ash corrected
[3]ADFom acid detergent fibre, ash corrected
[4]ADL acid detergent lignin
[5]ADIA acid detergent insoluble ash (a measure for abrasives)

Table 2. Tooth growth and wear (in mm per week) in guinea pigs (*Cavia porcellus*) reported in the literature and from this study

Study	Method	Diet	Upperincisor		Lowerincisor	
			Growth	Wear	Growth	Wear
(Shadle et al. 1938)	nm	nm	1.7-1.9	1.7-1.9	2.0-2.4	2.0-2.4
(Kamphues 2001)	toothmark	nm	1.4-1.7		1.2-1.9	
(Harkness et al. 2010)	nm	nm	1.05-1.92		1.05-1.92	
this study	tooth mark	L	1.38	1.09	1.91	1.71
		G	1.36	1.10	1.89	1.75
		GR	1.34	1.15	2.05	1.97
		GRS	1.51	1.20	2.12	2.08
		H	1.74	2.04	2.21	1.75

nm not mentioned; diets used in this study: complete pelleted diets (lucerne L, grass G, grass & rice hulls GR, grass & rice hulls & sands GRS) and grass hay (H)

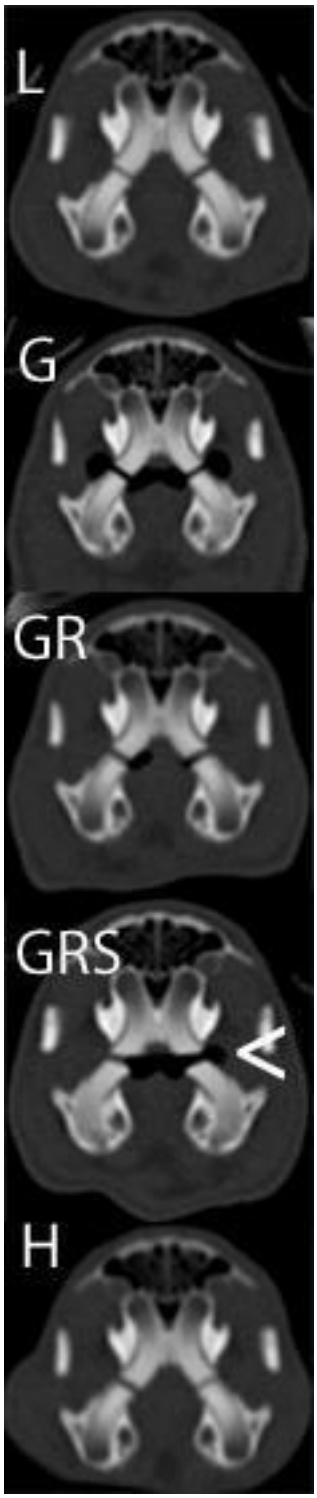
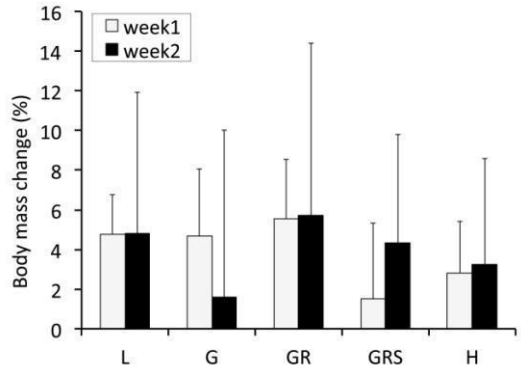
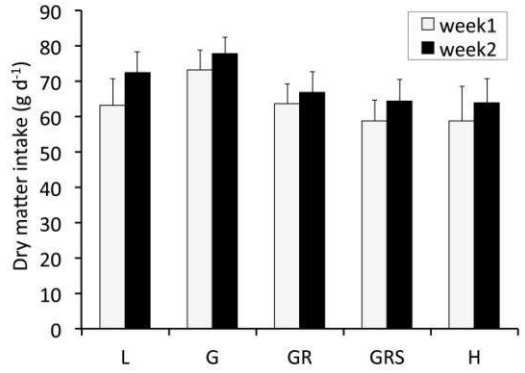


Figure 1. Cranial CT scans at the level of the first upper molar (M1) after 2 weeks on a given diet in guinea pigs(*Cavia porcellus*) fed pelleted diets of lucerne (L), grass (G), grass and rice husks (GR), grass, rice husks and sand (GRS), and whole grass hay (H). Note the decreased (nearly horizontal) tooth angle of M1 on GRS (white arrow).

a



b



c

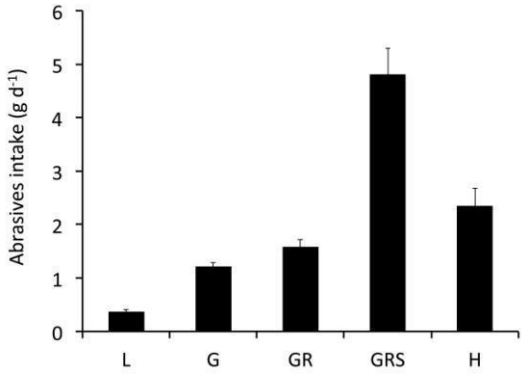


Figure 2. a) Body mass change (in %), b) dry matter intake (in g d^{-1}), and c) abrasives intake (measured as acid detergent insoluble ash, in g d^{-1}) in guinea pigs (*Cavia porcellus*) fed pelleted diets of lucerne (L), grass (G), grass and rice husks (GR), grass, rice husks and sand (GRS), and whole grass hay (H). For a) and b), data are given individually for the first and second week on the respective diet.

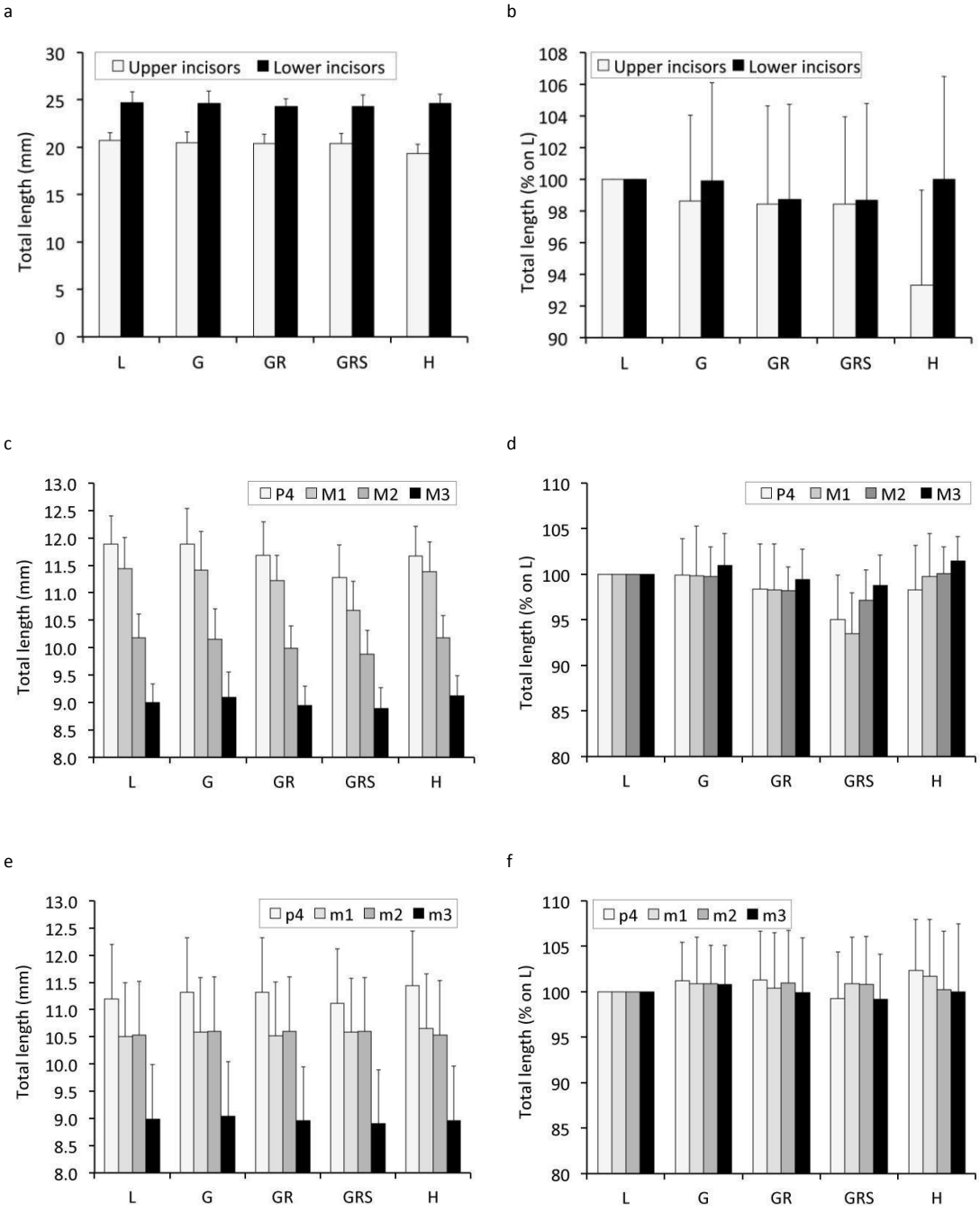


Figure 3. Mean total tooth lengths (for right and left teeth together) as measured on CT scans for ab) upper and lower incisors, cd) upper cheek teeth and ef) lower cheek teeth, expressed as an absolute measure (in mm) in a, c, e, and as a proportion of each individual's tooth on the lucerne diet in b, d, f in guinea pigs(*Cavia porcellus*) fed pelleted diets of lucerne (L), grass (G), grass and rice husks (GR), grass, rice husks and sand (GRS), and whole grass hay (H).

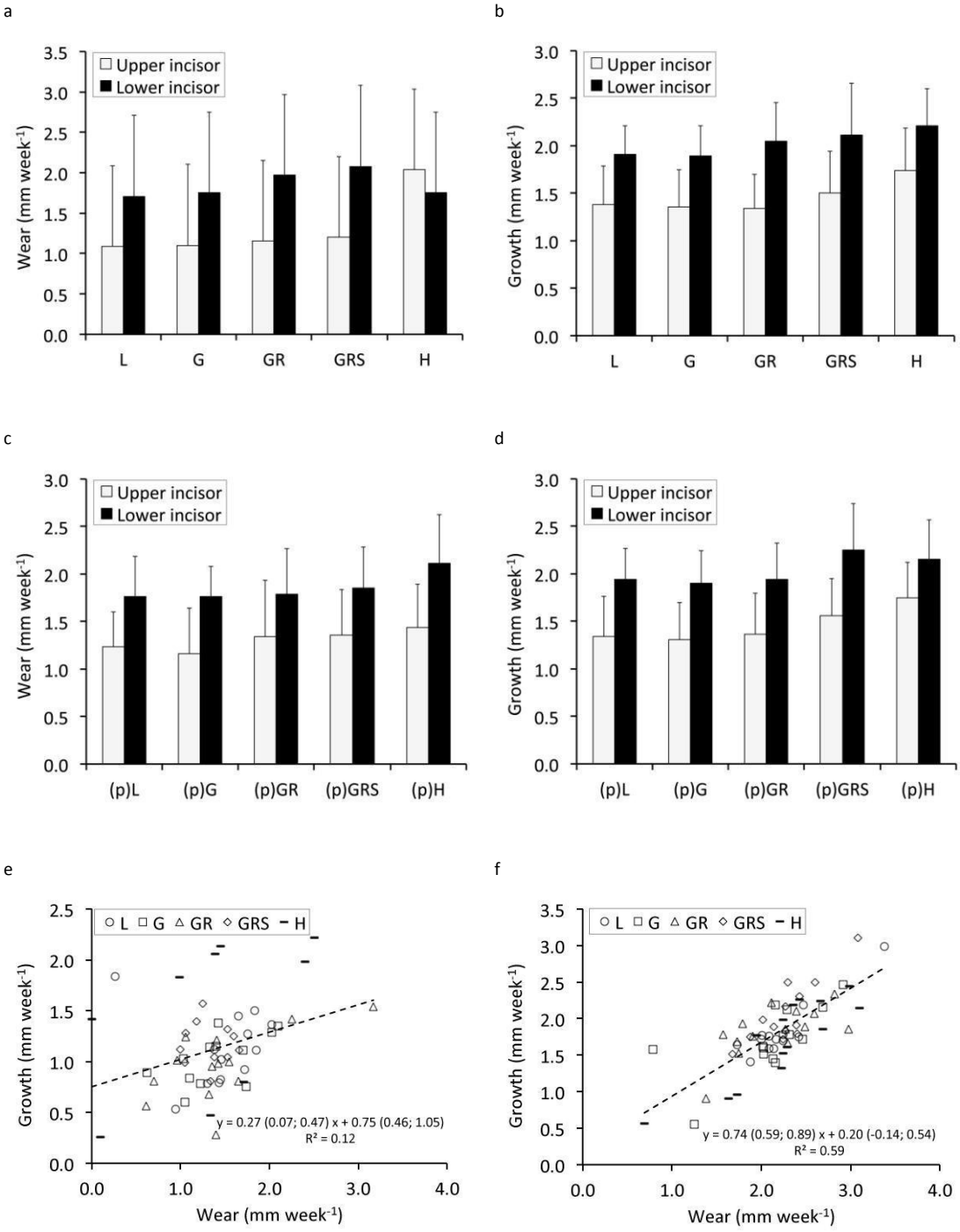


Figure 4. a) Mean wear and b) growth (in mm per week) and of the upper and lower incisors in guinea pigs (*Cavia porcellus*) fed pelleted diets of lucerne (L), grass (G), grass and rice husks (GR), grass, rice husks and sand (GRS), and whole grass hay (H); c) mean wear and d) growth of the incisors in relation to the diet fed the week preceding the measurement (on the 'previous' diet); and the relationship of wear and growth in e) upper incisor, f) lower incisor. Regression equations given with 95% confidence intervals of parameter estimates.

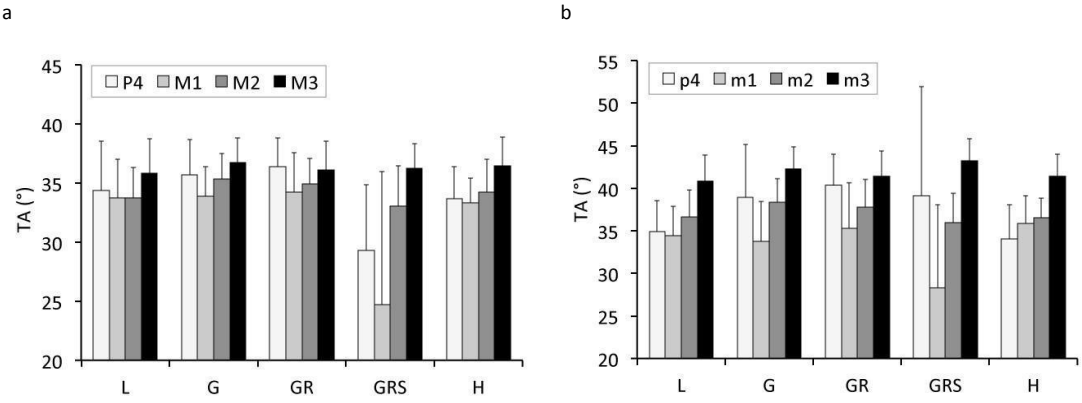


Figure 5. Tooth angle (TA, cf. Fig. 1) of a) upper and b) lower cheek teeth in guinea pigs (*Cavia porcellus*) fed pelleted diets of lucerne (L), grass (G), grass and rice husks (GR), grass, rice husks and sand (GRS), and whole grass hay (H).

Supplementary material

Tooth length and incisal wear and growth in guinea pigs (Cavia porcellus) fed diets of different abrasiveness

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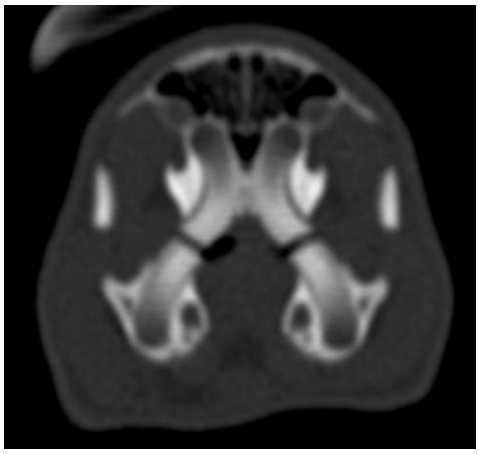
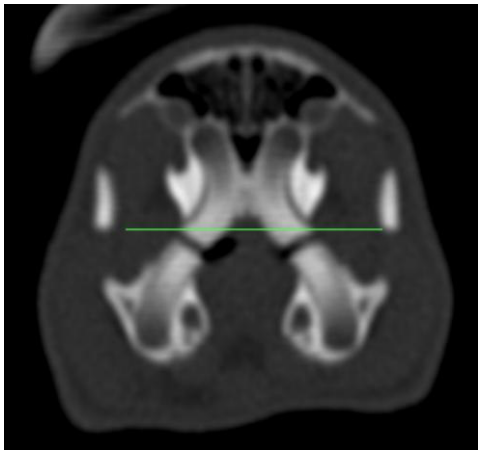
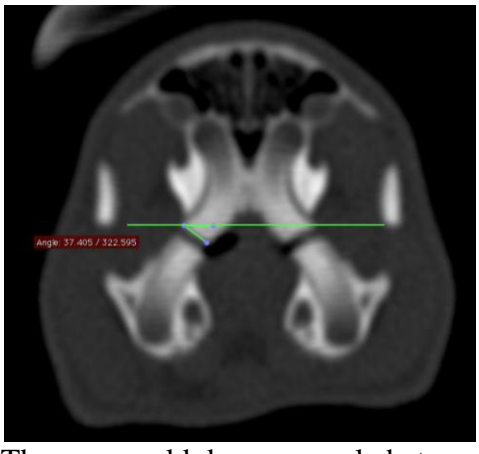
Tooth angle Measurement (=TA)



Sagittal plane of M1 in upper jaw



We drew a helping line between the buccal edge of both upper cheek teeth first.



Then we could draw an angle between tooth surface and helping line using a function which automatically measured the angle.

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